The Contribution of Fear Conditioning to Pathological Anxiety:

An Investigation of Conditioned Fear Generalization in OCD Traits and PTSD

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Dedication

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Abstract

A review of the literature demonstrates a lack of research on fear-generalization processes in many anxiety disorders including obsessive-compulsive disorder (OCD) and posttraumatic stress disorder (PTSD). Chapter 2 represents the first study that attempted to investigate the generalization of conditioned fear in individuals with obsessive-compulsive traits using startle EMG and behavioral measures. The results of this study demonstrated that individuals with high levels of Threat Estimation as measured by the Obsessive Beliefs Questionnaire (OBQ-44) displayed overgeneralization of fear responses to a greater range of stimuli resembling the danger cue than those with low levels of Threat Estimation. In addition, despite etiological theories proposing that fear conditioning and overgeneralization of fear play prominent roles in the development and maintenance of PTSD, little research had been done on the neurobiological mechanisms that contribute to fear conditioning processes in PTSD patients and none have been specifically conducted on generalization. Chapter 3 investigated the neurobiological substrates associated with the overgeneralization of conditioned fear in PTSD patients using behavioral, skin conductance, and functional magnetic resonance imaging (fMRI) measures. This study provides evidence that PTSD patients demonstrate overgeneralization of conditioned fear in the dorsal medial prefrontal cortex, bilateral insula, left and right caudate, left inferior parietal lobule, and right superior frontal gyrus. This body of work provides novel evidence regarding the generalization of conditioned fear in OCD and PTSD.

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Chapter 1: The Contribution of Fear Conditioning to Pathological Anxiety

An extensive literature has developed on the role of fear conditioning in the development of some forms of pathological anxiety. In fear-conditioning, conditioned fear occurs when an aversive unconditioned stimulus (US) is paired repeatedly with a neutral stimulus (conditioned stimulus or CS) leading the neutral stimulus to elicit anxiety associated with the anticipation of the aversive stimulus (conditioned response or CR). This literature dates back to the classical conditioning studies conducted by Ivan Pavlov (1927) and John B. Watson and Rosalie Rayner (1920). Although Watson and Rayner's (1920) Little Albert study demonstrated the pathologic effects of conditioned fear on human behavior, much of the early work in this area focused on learning in animals.

Additionally, the literature on fear conditioning in anxiety disorders remained primarily theoretical rather than experimental for many years. During that time, a number of theories were developed to explain the role of conditioned fear in the etiology of anxiety pathology. Etiological accounts of pathological anxiety have focused on abnormalities in a number of processes including: overly strong acquisition of conditioned fear responses, failure to inhibit fear responses to conditioned safety, resistance to extinguish fear responses, actively avoiding stimuli that would lead to conditioned fear responses, impaired ability to learn cues that predict danger, and generalizing conditioned fear to stimuli that resemble the original threat.

An increase in the number of studies on fear conditioning in humans in recent years has been suggested to be due to the development of more sophisticated

conditioning models that refine our understanding of fear and anxiety (Mineka & Zinbarg, 1996) and to the identification of specific brain regions associated with fear conditioning in animals and humans (Lissek et al, 2005). As a result of this renewed interest, there have been a number of studies in recent years on fear conditioning in anxiety patients. This chapter will provide an integrative review of fear-conditioning theories and research related to specific anxiety disorders to elucidate the relationship between fear conditioning and pathological anxiety symptoms and to identify gaps of knowledge in the literature. Specifically, this review will focus on research related to posttraumatic stress disorder (PTSD) and obsessive-compulsive disorder (OCD) and chapters 2 and 3 will report original research investigating fear-conditioning processes in each of these disorders.

Associative Fear Learning Theories

Associative fear learning refers to the association of a neutral stimulus with an aversive event that lead that neutral stimulus to trigger a fear response even when encountered without the aversive outcome. In contrast, non-associative learning is not based on conditioning; rather, changes in a behavioral response to a stimulus occur in the absence of positive or negative outcomes. For instance, through repeated exposure, an individual can habituate or become sensitized to a stimulus. In the literature on pathological anxiety, the number and diversity of associative fear learning theories has surpassed non-associative learning accounts. This section will provide an overview of the prominent fear conditioning theories that have been proposed to be related to anxiety

disorders. Following this, the literature on fear-conditioning processes in PTSD and OCD will be reviewed.

Failure to Extinguish

Extinction refers to a reduction in a previously learned response or CR following the repeated presentation of the CS in the absence of the US with which it was previously paired. Initially, it was proposed that extinction training caused the subject to "unlearn" or to erase the previously acquired association between the CS and US (Mackintosh, 1975; Rescorla & Wagner, 1972; Wagner & Rescorla, 1972). However, it is now accepted that extinction results in new learning rather than unlearning. During extinction, each time the CS is paired without the aversive stimulus, the subject is given the opportunity to re-encode information about the previously learned association. In other words, the subject encodes new information (the stimulus is safe) which competes for activation with the previously learned association (the stimulus is dangerous) and leads to the inhibition of the fear response to that stimulus (Bouton, 1993; Pearce, 1994; for a review, see Bouton, 2004). Additionally, extinction is not the result of forgetting. The passage of time alone is not sufficient for extinction to occur; extinction requires the presentation of the CS in the absence of the US (for an overview on extinction, see Myers & Davis, 2007).

Deficits in the ability to extinguish the fear response in the absence of danger can lead to the maladaptive persistence of anxiety symptoms, which has been suggested to be an essential feature of many anxiety disorders. Resistance to extinction may occur as the result of one of three processes: enhanced conditionability, deficits in inhibitory learning,

or "incubation." The enhanced conditionability account of resistance to extinction by Pitman and colleagues suggests that anxiety patients are characterized by enhanced acquisition of the CR and subsequent resistance to extinction (Orr et al., 2000; Peri et al., 2000). In other words, anxiety patients are predisposed to acquire the fear response to a greater degree than those without anxiety and this strongly formed association then makes extinction more difficult. The enhanced conditionability hypothesis is supported by the finding that fear conditioning is acquired more strongly in PTSD patients (Orr et al., 2000).

While the enhanced conditionability account focuses on abnormal levels of fear acquisition, a second theory proposes that deficits in inhibitory learning account for resistance to extinction (Davis, Falls, & Gewirtz, 2000; Jovanovic & Ressler, 2010). According to this account, anxious and nonanxious individuals show the same levels of acquisition of fear learning but deficits in extinction (inhibitory) learning result in failure to extinguish the fear response in anxiety patients. A deficit in the ability to learn new associations (the stimulus is now safe) and inhibit the CR would cause anxiety patients to have difficulty suppressing their fear response during extinction. The inhibitory learning theory is supported by research showing normative levels of conditioned fear acquisition but failure to suppress the learned fear response during extinction in anxiety patients (Howe, 1957; Pitman & Orr, 1986). These two theories (enhanced conditionability and deficits in inhibitory learning) can be described as the result of an overly strong acquisition memory and an insufficiently strong extinction memory, respectively (Lissek & Grillon, 2012).

A third process by which resistance to extinction could occur was proposed by Eysenck (1976), who suggested the "incubation of fear" theory. According to this account, the CR creates an internal state of fear that functions as the US, thereby impeding extinction. Specifically, Eysenck suggests that anxious individuals experience the CR as significantly more "nocive" or unpleasant than non-anxious individuals. This arousal is suggested to be substantial enough to serve as a replacement for the US which not only results in resistance to extinction but has been suggested to "incubate" or enhance the CR over time. There are currently few studies that have directly tested the incubation hypothesis.

In addition to resistance to extinction, retention of the extinguished fear response is also relevant to research on fear conditioning and anxiety pathology. Following conditioned fear acquisition and extinction training, a retention test can be administered after a delay to determine the degree to which extinction learning persists over time. There is some evidence suggesting that abnormalities in the retention of extinction learning may be important to anxiety pathology. For example, anxiety disorder patients show impaired extinction recall after a delay following otherwise normative extinction learning (Milad et al., 2008; Milad et al., 2009). However, additional research on extinction retention is needed before inferences can be made regarding the contribution of this process to the maintenance of anxiety symptoms.

An extinguished conditioned response that reappears during retention tests can occur under several conditions, which are referred to as the reinstatement, renewal, or spontaneous recovery of the fear response (Myers & Davis, 2007). Reinstatement refers

to the re-emergence of the extinguished fear response during unanticipated presentations of the US. Renewal refers to the reappearance of the extinguished CR when the retention test occurs in a different context than the extinction training. Spontaneous recovery refers to the unanticipated re-emergence of the fear response following the passage of time without further extinction training. For a description of the typical experimental paradigm used in each type of retention test, see the fear conditioning paradigms section or refer to Myers and Davis (2007). We might expect that anxiety patients would show greater reinstatement, renewal, and/or spontaneous recovery of the fear response compared to non-anxious individuals.

Mowrer's Two-Stage Learning/Avoidance

Mowrer's two stage learning theory is closely associated with the failure to extinguish hypotheses. In this theory, avoidance is the primary mechanism by which subjects fail to extinguish maladaptive fear responses (Mowrer, 1947; 1960). In other words, a subject who avoids fear-related stimuli will never experience the exposure to the CS that is necessary for extinction to occur or will experience insufficient exposure to facilitate extinction. This theory is based on the extinction principle that subjects need to be given the opportunity to re-encode information about the previously learned CS-US association before the fear response can be extinguished. The anxiety patient has encoded the information that a stimulus is dangerous and without the opportunity for new learning to take place, this association receives no competition for activation with an inhibitory extinction memory (Lissek & Grillon, 2012). Because, according to this perspective, avoidance is the primary mechanism for extinction failure, it can be inferred from this

theory that anxious individuals would have normative levels of fear acquisition and possibly normative ability to extinguish the fear response. However, anxiety patients deprive themselves of the opportunity to extinguish the fear response through avoidance.

Mowrer's two stage learning theory is supported in part by clinical research showing the effectiveness of exposure therapy in treating some types of anxiety disorders (Barlow, 2002; Mineka & Thomas, 2005; Parsons & Rizzo, 2008; Powers & Emmelkamp, 2008; Powers et al., 2010). When fears have been conditioned, the basis of exposure therapy is extinction: presenting the CS repeatedly without the aversive stimulus until the fear response declines. The importance of avoidance in maintaining anxiety symptoms is supported by research showing persistence of the conditioned fear response in participants who are given the opportunity to actively avoid the CS during extinction training (Lovibond et al., 2009). These results are applicable to the clinical setting where failure to reduce anxiety is observed in patients who actively avoid exposure by refusing treatment (noncompliance) or who use avoidance strategies (e.g., distraction) during therapy sessions (Grayson, Foa, & Steketee, 1982). Importantly, exposure therapy can only be compared to extinction learning if the original fear was conditioned; otherwise, exposure therapy is more akin to habituation.

Failure to Inhibit Fear

The theory that pathological anxiety is associated with deficits in inhibitory learning during extinction is also applicable beyond the extinction literature. The impaired ability to inhibit a conditioned fear response has been suggested by Davis and colleagues (2000) and Jovanovic and Ressler (2010) to affect symptoms of anxiety more

broadly than simply resistance to extinction. Conditioned fear inhibition involves learning to discriminate between safety and danger cues in the environment and using safety cues as a signal to suppress the fear response. Deficits in inhibitory fear learning would lead to the expression of fear responses in the absence of danger, not only during extinction but during any process that relies on conditioned fear inhibition.

The validity of the conditioned fear inhibition theory is supported by studies demonstrating larger fear responses to learned safety cues in anxiety patients (Grillon & Morgan, 1999; Jovanovic et al., 2010; Orr et al., 2000; Peri et al., 2000) and in non-clinical subjects with high levels of self-reported anxiety (Grillon & Ameli, 2001). However, studies finding that anxiety patients show enhanced fear responses to safety cues are mostly limited to PTSD patients. Some studies have been able to show a greater subjective expectation that the safety cue would be followed by an aversive stimulus in anxious or "neurotic" patients (Clum, 1969) and in individuals with social phobia (Hermann et al., 2002). An excessive fear response to safety cues is also predicted by the over-generalization of conditioned fear theory; however, the inhibition and the overgeneralization theories differ in their focus with the former concerned with inhibitory processes and the latter concerned with excitatory processes.

Associative Learning Deficits

While Pitman and colleagues' conditionability theory would predict that anxious subjects would be characterized by enhanced acquisition of the CR, a theory by Grillon (2002) proposed that anxious subjects would show the opposite: impaired acquisition of the conditioned fear response. Specifically, this theory is based on the notion that an

essential trait of anxiety is apprehension about unpredictability. This perceived sense of unpredictability could arise if anxious individuals are unable to learn which cues signify fear and which cues do not. Classical fear conditioning paradigms rely on predictability; conditioning does not occur if contingencies between stimuli are random. Therefore, fear conditioning provides an opportunity to test whether anxious individuals are aware of this predictability or whether they show deficits in learning fear associations.

If anxious individuals are less able to learn the association between the CS and US, the subject's ability to predict danger and safety cues will be impaired, leading to what Grillon (2002) predicts will be a chronic state of anxiety. In other words, this theory postulates that the primary deficit leading to pathological anxiety is the inability of anxiety patients to effectively learn which specific cues indicate danger, leaving the danger unpredictable. Additionally, the inability to associate danger with a specific feature of the context will lead the person to associate the entire context with danger, resulting in contextual anxiety. Transference of fear to the context is supported by research showing that unpredictable presentations of the CS and US increase contextual fear (Grillon & Davis, 1997).

There are few studies that have sought to substantiate the hypothesis that pathological anxiety is related to associative learning deficits. Grillon (2002) was able to provide evidence supporting this theory by demonstrating that subjects with deficits in explicit cue fear conditioning (subjects who are unaware of the CS-US relationship) show increased physiological indicators of anxiety during the session and greater avoidance, as measured by failure to return for a second session. However, this study used healthy

participants, not anxiety patients. An additional study by Grillon et al. (2009) found that anxiety patients show greater fear responses during unpredictable presentations of the US, supporting the link between unpredictability and enhanced anxiety. Conversely, others do not find impaired associative learning in anxiety patients (Burriss, Ayers & Powell, 2007).

Overgeneralization of Conditioned Fear

The generalization of conditioned fear refers to the transfer of the conditioned fear response to stimuli that resemble the original CS. Conditioned fear generalization was first demonstrated by Watson and Rayner (1920) in the "Little Albert" studies which showed that conditioning a child to fear a white rat led to the generalization of this fear to many other white furry animals and objects. Others have suggested the importance of the generalization of conditioned fear in the development and maintenance of anxiety disorders (Bouton, Mineka, & Barlow, 2001; Goldstein & Chambless, 1978; Mineka & Zinbarg, 2006). Intuitively, it would seem adaptive to fear animals or objects that resemble known dangers. However, as with other symptoms of anxiety, the excessive generalization of fear can become maladaptive when individuals continually perceive danger in harmless stimuli.

According to the generalization theory of conditioned fear, anxious individuals may be predisposed to have a lower threshold for threat reactivity; therefore, the degree of resemblance to the CS required to elicit the fear response would be reduced in anxiety patients (Lissek et al., 2010; Lissek & Grillon, 2012). In other words, hyper-reactivity to threat would lead to the enhanced perception of danger in a greater range of benign

stimuli resembling the original threat. The generalization theory of conditioned fear would predict normative levels of acquisition of conditioned fear to danger cues but greater expression of that fear to a larger number of stimuli resembling the CS.

As mentioned previously, enhanced fear responses to safety cues would be predicted by both the overgeneralization of conditioned fear theory and the conditioned fear inhibition theory. Multiple studies have found that pathological anxiety is associated with exaggerated fear responses to safety cues (Clum, 1969; Grillon & Ameli, 2001; Grillon & Morgan, 1999; Hermann et al., 2002; Jovanovic et al., 2010; Orr et al., 2000; Peri et al., 2000), which could be taken as evidence supporting the overgeneralization theory. Additionally, studies using a paradigm designed specifically for testing the generalization of conditioned fear (see Lissek et al., 2008) found that anxiety patients show an enhanced fear response to a larger range of stimuli resembling the CS than healthy controls (Lissek et al., 2010; Lissek et al., under review), which supports the notion that anxiety is associated with overgeneralization of conditioned fear.

Etiological Theories and Fear Conditioning Research in PTSD and OCD

A meta-analysis by Lissek et al. (2005) found evidence that anxiety disorder patients are characterized by enhanced acquisition of the conditioned fear response and resistance to extinction in comparison to controls. However, the authors note that the contribution of associative fear learning likely varies by type of anxiety disorder, suggesting the importance of conducting studies investigating disorder-specific hypotheses. A review of the etiological theories and related fear conditioning studies associated with PTSD and OCD provides a better understanding of the relative

contribution of associative fear learning to these disorders. For each disorder, a brief overview of the relevant etiological theories will be provided and this information will be integrated with the findings from fear conditioning studies. A comprehensive review of the fear conditioning studies related to PTSD and OCD will be used to demonstrate the important role of associative fear learning in the development and/or maintenance of these disorders.

Posttraumatic Stress Disorder

Posttraumatic stress disorder develops in some individuals following an extreme traumatic stressor and is characterized by feelings of intense fear, helplessness, or horror, re-experiencing the event, increased arousal, and avoidance of stimuli associated with the traumatic event (PTSD; American Psychiatric Association, 2000). Etiological models of PTSD have focused on a variety of theoretical frameworks regarding the biological and psychological vulnerabilities that may contribute to PTSD symptoms (for an overview, see Jones & Barlow, 1990). Two theoretical perspectives that are relevant to the current discussion are cognitive/information processing models and behavioral models. The cognitive/information processing perspective focuses on the "fear structure," a memory network that stores information about fear relevant stimuli, appropriate responses, and interpretation of stimuli and responses. The significance and intensity of a traumatic event causes the fear structure to be more easily activated in PTSD patients which results in symptoms such as re-experiencing the event and avoidance of stimuli associated with the traumatic incident (Jones & Barlow, 1990).

Similarly, the behavioral perspective also implicates the significance and intensity of a traumatic event as the cause of PTSD symptoms. According to this perspective, fear conditioning is the essential process that leads to the development of psychopathology to trauma. In a fear conditioning explanation of trauma psycholpathology, individuals who experience an aversive event are then conditioned to fear stimuli related to the traumatic event. When this conditioned fear is excessive and/or does not subside after the trauma, a diagnosis of PTSD may be given. In PTSD, the traumatic event is aversive enough (involves actual or threatened death or serious injury to self or others) to create a conditioned fear response after only a single experience. Symptoms of PTSD such as hypervigilance and enhanced startle reactions are thought to be directly related to this conditioning process. A fear conditioning explanation is especially relevant to PTSD since, unlike many other anxiety disorders, there is typically a direct association between a conditioning (traumatic) experience and subsequent fear-responding to trauma-relevant stimuli.

Research using fear conditioning paradigms supports this etiological account of PTSD. Fear conditioning accounts of PTSD have focused on abnormalities in the acquisition, extinction, associative learning, inhibition, overgeneralization, or avoidance of conditioned fear. Empirical evidence for each of these theories is mixed. Some studies find stronger acquisition of the fear response in PTSD patients (e.g Orr et al., 2000) while most others do not (Blechert et al., 2007; Grillon & Morgan, 1999; Milad et al., 2008; Milad et al., 2009; Peri et al., 2000), suggesting that overly strong acquisition of the conditioned fear response may not contribute substantially to PTSD symptoms. In terms

of the failure to extinguish theory, there is evidence that PTSD patients are characterized by resistance to extinction in some studies (Blechert et al., 2007; Norrholm et al., 2011; Orr et al., 2000; Peri et al., 2000; Wessa & Flor, 2007) but not others (e.g., Grillon & Morgan, 1999). Abnormalities in retention of extinction learning may be relevant to PTSD since some studies have found that PTSD patients show impaired extinction recall after a delay following otherwise normative extinction learning (Milad et al., 2008; Milad et al., 2009). However, additional research on extinction retention is needed to replicate these findings.

In support of the associative learning deficits theory, there is some evidence that PTSD is associated with sustained contextual anxiety (Grillon et al., 2009) which may constitute a risk factor for developing PTSD (Pole et al., 2009). However, others do not find associative learning deficits in PTSD patients (Burriss, Ayers, and Powell, 2007). A number of studies find enhanced fear responses to safety cues in PTSD patients using a discrimination learning paradigm (Grillon & Morgan, 1999; Grillon et al., 1998; Jovanovic et al., 2010; Orr et al., 2000; Peri et al., 2000), which supports both the inhibition of conditioned fear theory and, due to the perceptual similarity of the CS+ and CS-, the overgeneralization theory. Both theories would predict enhanced fear responses to safety cues either due to an inability to inhibit the fear response or the generalization of conditioned fear to the safety cue. However, based on the existing research, it is not possible to determine the relative contributions of inhibitory learning or overgeneralization to the maintenance of PTSD symptoms. Lastly, although the

avoidance theory predicts that conditioned fear will lead to the instrumental avoidance of trauma relevant stimuli, this hypothesis has not yet been systematically tested.

A well developed conceptual framework based on conditioned fear and empirical support from a number of fear conditioning studies suggests that an associative fear learning account of PTSD is a promising endeavor. What is now needed is additional research that can clarify the relative importance of the various fear conditioning theories in the development of PTSD. For example, research in this area would benefit from studies investigating the importance of specific fear conditioning theories that have been understudied, such as avoidance and extinction retention. Additionally, further research is needed to dissociate the contributions of the inhibitory learning theory from the overgeneralization theory.

Obsessive-compulsive Disorder

Obsessive-compulsive disorder is characterized by obsessions (intrusive thoughts) that cause significant anxiety or distress and/or compulsions (repetitive acts) that are used to reduce anxiety (OCD; American Psychiatric Association, 2000). The most common themes for obsessions are contamination, repeated doubts (e.g., whether the door is locked), a need to have things in a particular order, aggressive or horrific impulses (e.g., to hurt a family member), and sexual imagery. Compulsions are used to prevent or reduce anxiety or distress and are characterized by repetitive behaviors (e.g., hand washing, checking, ordering) or mental acts (e.g., praying, counting). Etiological models of OCD propose a number of biological and psychological vulnerabilities that may contribute to OCD symptoms (for an overview, see Abramowitz, Taylor, & McKay, 2009).

One of the more widely accepted psychological models of OCD is the cognitive-behavioral theory which proposes that unwanted cognitive intrusions are appraised by OCD patients as highly unacceptable or immoral, are believed to be personally important, and/or are thought to pose a threat for which the individual is personally responsible (Abramowitz, Taylor, & McKay, 2009). Central to this perspective is the use of compulsions to avoid fear and anxiety associated with obsessions. The cognitive-behavioral model proposes that compulsions become persistent because they provide negative reinforcement (temporary reduction in distress). Furthermore, compulsions serve as an avoidance strategy that prevents the individual from learning that obsessions will not result in harmful consequences.

Etiological theories do not typically implicate conditioned fear as the origin of obsessive-compulsive disorder; however, fear conditioning processes may still contribute to OCD symptoms. For example, overgeneralization may be pertinent to OCD. Anecdotal evidence for overgeneralization is apparent in descriptions of OCD symptoms; for example, an individual with a fear of contamination from a certain object may then generalize that fear to other objects or people that have been in contact with the original object. Additionally, patients with OCD have been suggested to overestimate threat levels (Myers et al., 2008). A tendency toward overestimating threats is an important precursor to conditioned generalization and may predict overgeneralization in OCD. Furthermore, patients with OCD show stronger amygdala involvement for both OCD-related images and general aversive images which has been interpreted as evidence of overgeneralization of the emotional response to non-symptom specific stimuli (Simon et al., 2010).

There is currently little research on the generalization of conditioned fear in individuals with OCD. One study demonstrated that OCD patients show faster eyeblink conditioning relative to controls (Tracy et al., 1999); however, eyeblink conditioning is not equivalent to fear conditioning. Because of the lack of fear conditioning studies using OCD patients, inferences cannot be made about the role of associative fear learning in the maintenance of this disorder. Investigation of the generalization of conditioned fear in individuals with OCD is warranted. Additionally, research examining the acquisition, inhibition, and extinction of conditioned fear in OCD patients is needed. Associative learning deficits have also been understudied in OCD patients. Another possible direction in the research on fear conditioning in OCD would be to investigate instrumental avoidance in OCD patients since avoidance is thought to be an important feature of the cognitive-behavioral model of OCD.

Neurobiological Mechanisms Associated with PTSD and OCD: Relation to Fear Conditioning

The purpose of this section is to review the neurobiological mechanisms implicated in PTSD and OCD and to integrate these findings with the fear conditioning literature. In particular, for each disorder there are very few (if any) fear conditioning studies that were conducted using imaging methods. Therefore, the following discussions for each anxiety disorder will begin with a broader review of patient-control differences in brain activation during a variety of tasks that extend beyond the fear conditioning literature. This will allow for an evaluation of the neural processes that may be abnormal in each anxiety disorder, which can be used to make inferences about common neural

deficits, some of which may be associated with abnormalities in fear conditioning processes. Before discussing disorder-specific imaging research, a brief overview of the general neural mechanisms associated with fear conditioning will be provided.

Neural structures that are thought to be important for fear conditioning include the amygdala, insula, hippocampus, the bed nucleus of the stria terminalis (BNST), and the ventral medial prefrontal cortex (Davis & Shi, 1999; Jovanovic & Ressler, 2010; Kim & Fanselow, 1992; Lissek et al., 2013; Phillips & LeDoux, 1992). The amygdala, part of the limbic system located in the medial temporal lobe, is thought to be involved in the expression of fear (Blaire et al., 2001; for a review, see Phan et al., 2002). The amygdala is comprised of several nuclei including the central nucleus, basolateral nucleus, and other nuclei. Lesions to the central nucleus of the amygdala inhibit fear-potentiated startle and freezing in rodents (Davis et al., 1982; LeDoux, 1992) and temporal lobectomy eliminates fear-potentiated startle in humans (Funayama et al., 2001). Additionally, fMRI studies show amygdala activation to the presentation of fear cues during fear conditioning (Bremner et al., 2005; Knight, 2005; LaBar et al., 1998; Phelps et al., 2004) and during threat of shock without actual administration of shock (Phelps et al., 2001).

The basolateral nucleus is thought to be important for forming CS-US associations during fear acquisition and for projecting to the central nucleus to activate or inhibit fear responses. The BNST is part of the extended amygdala and is associated with non-specific anxiety and depressive symptoms (Jovanovic & Ressler, 2010). The amygdala directly and indirectly receives sensory information from various brain regions including the thalamus and associative inputs from the hippocampus (Ehrlich et al.,

2009). The hippocampus is thought to be important for the learning and consolidation of fear-related memory (Lissek & Grillon. 2012). Furthermore, the insula is associated with processing fear and disgust and increased activation of the insula has been documented during fear-conditioning (Lissek et al., 2013). Additionally, the ventral medial prefrontal cortex (vmPFC) has been shown to be important in inhibitory learning during extinction (Morgan, Romanski, & LeDoux, 1993) and may have inhibitory effects on the amygdala (Grace & Rosencrantz, 2002; Phelps et al., 2004). Pathological anxiety is thought to be associated with deficits in this complex neural circuitry (Jovanovic & Ressler, 2010). However, functional abnormalities in each of these regions vary between anxiety disorders.

Posttraumatic Stress Disorder

Research on the neural processes associated with PTSD symptoms has shown abnormalities in functioning in several brain regions. There is evidence of abnormalities in activation of the insula, amygdala, dorsal medical prefrontal cortex (dmPFC), rostral anterior cingulate cortex, hippocampus, and ventral medial prefrontal cortex of PTSD patients (Shin & Handweger, 2009; for a review, see Liberzon & Sripada, 2008). As mentioned previously, increased activation of the insula, amygdala, and dmPFC and decreased activation of the hippocampus and ventral medial prefrontal cortex are thought to be important in fear conditioning (e.g., Lissek et al., 2013). Non-fear conditioning studies using single photon emission computed tomography (SPECT), PET, or fMRI find greater levels of insula, amygdala, and/or dmPFC activation while listening to trauma-related scripts (Liberzon et al., 2007; Osuch, 2001; Rauch et al., 1996) and sounds

(Liberzon et al., 1999), while viewing trauma-related imagery (Bremner, et al., 1999; Shin, et al., 1999) and words (Protopopescu et al., 2005), and when viewing fearful faces (Rauch et al., 2000; Shin et al., 2005). Furthermore, non-fear conditioning studies have also documented decreased activation of the ventral medial prefrontal cortex in PTSD patients during symptom provocation (Bremner, et al., 1999; Osuch, 2001). The importance of these regions in PTSD patients is also supported by a meta-analysis by Etkin and Wager (2007). However, none of these studies employed a fear conditioning paradigm.

Studies using fear conditioning paradigms support the finding of abnormalities in these brain areas in PTSD. Bremner et al. (2005) found that PTSD patients showed increased activation in the left amygdala during fear acquisition and decreased activation in the anterior cingulate during extinction relative to controls. A study using discrimination fear conditioning found increased amygdala activation in PTSD patients relative to controls during extinction learning but decreased hippocampus and ventromedial prefrontal cortex activation during extinction recall 24 hours later (Milad et al., 2009). Furthermore, a study using a discrimination fear conditioning experiment with threat of shock (not actual shock) found that PTSD patients show decreased ventromedial prefrontal cortex activity during threat of shock compared to controls (Tuescher et al., 2011). Deficits in the ability of the vmPFC to inhibit amygdala activation have been suggested to contribute to abnormalities in associative fear learning in PTSD. The finding of excessive amygdala activation and decreased ventromedial prefrontal cortex activation

suggests that PTSD patients may be associated with abnormalities in inhibitory neural circuits.

Obsessive-compulsive Disorder

Obsessive-compulsive disorder is thought to be linked to abnormal metabolic activity in a cortical-striatal circuit that includes pathways from the orbital frontal cortex, the anterior cingulate/caudal medial prefrontal cortex, and the caudate nuclei of the basal ganglia (Lucey et al., 1997; Rauch & Baxter, 1998; Saxena et al., 1998; Wilson, 1998; for a review, see Graybiel & Rauch, 2000). This circuit has been show to be hyperactive in OCD patients using electroencephalography (EEG), positron emission tomography (PET), magnetoencephalolgraphy (MEG), and MRI studies (for a review, see Stein, 2000; 2002). There is also support for the role of the amygdala in the manifestation and/or maintenance of OCD symptoms. Several studies have found greater activation of the amygdala in response to symptom provocation in OCD patients (Breiter et al., 1996; van den Heuvel et al 2004, 2005; Simon et al., 2010). Other studies have also found differences in amygdala volume in OCD patients compared to controls (Szeszko et al 1999). There are currently no imaging studies using a fear conditioning paradigm in OCD patients; therefore, more research is required before inferences can be made about the neural mechanisms associated with fear conditioning processes in OCD.

Conclusion

A review of the literature on fear-conditioning processes in PTSD and OCD reveals a number of areas that would benefit from additional research. One notable gap in the literature is the lack of studies on the generalization of conditioned fear in PTSD,

despite etiological theories proposing that fear conditioning and overgeneralization of fear play prominent roles in the development and maintenance of this disorder. Additionally, little is known about the neurobiological mechanisms that contribute to fear generalization in PTSD. Some studies implicate the amygdala, insula, hippocampus, and ventral medial prefrontal cortex as possible regions of abnormal functioning in PTSD. However, it is important to note that various brain regions (e.g., the amygdala) are implicated in a number of diverse psychological processes besides fear conditioning. Therefore, conclusions about the brain mechanisms implicated in fear conditioning are tentative at best when no imaging studies employing a generalization paradigm exist. This highlights the need for additional imaging studies using a conditioned fear generalization paradigm with PTSD patients.

Furthermore, a review of the literature also demonstrates a paucity of research on fear-conditioning processes in OCD. Research outside of the fear conditioning literature suggests that those with OCD may overgeneralize threat; however, no studies to date have investigated conditioned fear generalization processes in OCD. The purpose of this dissertation is to elucidate the role of the generalization of conditioned fear in PTSD and OCD. Chapter 2 reports on original research examining conditioned fear generalization in individuals with obsessive-compulsive traits using startle EMG. Chapter 3 investigates the neurobiological substrates associated with the overgeneralization of conditioned fear in PTSD patients using functional magnetic resonance imaging (fMRI) as well as skin conductance and behavioral measures. And finally, chapter 4 will provide a general discussion of the implications of these studies and will discuss areas for future research.

Chapter 2: Generalization of Conditioned Fear and Obsessive-compulsive Traits

As shown by the literature reviewed in Chapter 1, the overgeneralization of conditioned fear is thought to be an important feature of pathological anxiety. Overgeneralization of conditioned fear has been associated with panic disorder (Lissek et al., 2010) and generalized anxiety disorder (GAD; Lissek et al., under review) and may be relevant to other anxiety disorders as well, such as obsessive-compulsive disorder (OCD). OCD is a chronic and debilitating disorder characterized by intrusive thoughts and repetitive acts to reduce anxiety (American Psychiatric Association, 2000). Anecdotal evidence for overgeneralization is apparent in descriptions of OCD symptoms. An individual with a fear of contamination from a certain object may then generalize that fear to other objects or people that resemble the original object. For example, an OCD patient who encounters a particularly unsanitary public restroom may develop a fear of contamination from using not only that one restroom but all public restrooms. The threat posed by the one restroom has been generalized to all restrooms despite safety cues in the environment (e.g., apparent cleanliness) that should inhibit the fear response to sanitary public restrooms.

Additionally, OCD has been linked to a tendency to overestimate threat (Abramowitz, Taylor, & McKay, 2009; Myers, Fisher, & Wells, 2008) and the Obsessive-Beliefs Questionnaire (OBQ-44) recognizes excessive threat estimation as one domain of symptoms related to OCD (Obsessive Compulsive Cognitions Working Group, 2005). Overestimation of threat refers to beliefs that one's environment is unsafe, despite evidence to the contrary. In the context of generalization, those high on threat

estimation should be over-reactive to stimuli resembling the danger cue even though the dissimilar part of the stimulus is actually a sign of safety. As such, a tendency toward overestimating threats may be an important precursor to conditioned generalization and may predict overgeneralization in OCD. Furthermore, neuroimaging research supports the association between OCD and the possible overgeneralization of fear responses. Patients with OCD show stronger amygdala involvement for both OCD-related images and general aversive images, which has been interpreted as evidence of "generalized emotional hyperresponsivity" to non-symptom specific stimuli (Simon, Kaufmann, Müsch, Kischkel, & Kathmann, 2010).

Despite the intuitive relationship between fear generalization and OCD, there is currently no research that systematically investigates the generalization of fear in individuals with obsessive-compulsive traits using fear conditioning paradigms. The purpose of the current study is to determine the degree to which individuals with obsessive-compulsive (OC) traits generalize conditioned fear when compared to healthy participants. In this study, participants completed a generalized fear conditioning task based on discriminative fear-conditioning as described in Lissek et al. (2010). In discriminative conditioning, two conditioned stimuli are presented, one that is paired with the unconditioned stimulus (referred to as the CS+ or danger cue) and one that is not paired with the unconditioned stimulus (CS- or safety cue). Within-subject effects are measured in discrimination conditioning as the difference in fear-potentiated startle amplitudes to the danger versus safety cues. The fear-potentiated startle response is the

reliable enhancement of the startle reflex when a person is in a state of fear (Grillon, Ameli, Woods, Merikangas, & Davis, 1991).

In addition to the presentation of CS+ and CS-, generalization stimuli forming a continuum of similarity between the CS+ and CS- are presented to test generalization effects. Specifically, this paradigm produces generalization gradients (slopes) where fear responses decrease as generalization stimuli become less similar to the conditioned danger cue (Lissek et al., 2008). Furthermore, these generalization gradients have been shown to differentiate between healthy participants and both panic disorder and generalized anxiety disorder (GAD) patients. Healthy participants show steep quadratic declines in fear responses to the generalization stimuli while responses in panic and GAD patients are characterized by linear, more gradual declines in fear responses to the generalization stimuli (Lissek et al., 2010, Lissek et al., under review). In other words, as stimuli become less similar to the danger cue, healthy participants are able to differentiate between the danger cue and approximations of the danger cue and their fear responses quickly decline. However, panic and GAD patients are less able to emotionally differentiate between the danger cue and its approximations; thus, they continue to show high levels of fear to stimuli that resemble the danger cue, suggesting overgeneralization of conditioned fear.

The current study hypothesizes that individuals with OC traits will show overgeneralization of conditioned fear compared to controls as measured by startle potentiation and self-report ratings. Specifically, healthy participants are predicted to show quadratic generalization gradients, suggesting normal, more precipitous declines in

conditioned responding as the presented stimulus differentiates from CS+. Participants with OC traits are predicted to show linear declines in generalization gradients, suggesting overgeneralization of conditioned fear. In particular, this study is interested in the overestimation of threat since high levels of Threat Estimation as measured by the OBQ-44 are predicted to be associated with the overgeneralization of conditioned fear.

Methods

Participants

Participants included 59 adults (38 females, 21 males) whose ages ranged from 18 to 30 years of age. Participants were selected based on their responses to the Obsessive-Compulsive Inventory- Revised (OCI-R), a 18-item questionnaire that measures six dimensions of OCD symptoms including washing, obsessing, hoarding, ordering, checking, and neutralizing (Foa et al., 2002). This scale can be used to screen for the frequency of obsessive-compulsive symptoms and to measure symptom severity using a 5-point Likert scale of subjective distress. The OCI-R has been shown to have adequate psychometric properties in both clinical and nonclinical samples (Fullana et al., 2005; Hajcak, Huppert, Simons, & Foa, 2004; Huppert et al., 2007).

A total of 470 undergraduates completed the Obsessive-Compulsive Inventory-Revised (OCI-R; Foa et al., 2002) using a secure online survey. From these students, 59 individuals met the criteria for this study and were recruited for the psychophysiological recording session based on their total OCI-R score. The present study adopts Foa et al.'s (2002) recommendation that a clinically significant cutoff is an OCI-R score of 21 or greater. Using this criterion, two similarly sized groups were selected: a high obsessive-

compulsive group (OCI-R ≥ 21) consisting of 28 individuals (16 females, 12 males) and a low obsessive-compulsive group (OCI-R ≤ 20) composed of 31 individuals (22 females, 9 males). Foa et al.'s (2002) recommended cutoff score of 21 on the OCI-R does not imply that an individual with a score of 21 or greater would be diagnosed with OCD; instead, a score of 21 or greater suggests that the participant endorses obsessive-compulsive symptoms to a greater extent than expected in a healthy sample. Table 2.1 shows the demographics for the high and low obsessive-compulsive groups. There were no between-group differences in age.

Table 2.1

Group characteristics for the high and low obsessive-compulsive groups

	Low Obsessive-Compulsive	**High Obsessive-Compulsive**
Sample size (females, males)	31 (22, 9)	28 (16, 12)
Age in years	20.68 (3.91)	21.29 (3.13)
OCI-R	5.63* (6.65)	30.21* (8.59)
OBQ-44	132.17* (32.07)	176.36* (33.10)
BDI	5.16* (5.72)	17.69* (8.93)
SAI	32.84* (9.08)	45.76* (9.12)

Note. Group means and (standard deviations) are reported; Significant group differences ($p < .05$) are denoted with an asterisk. OCI-R = the Obsessive-Compulsive Inventory-Revised; OBQ-44 = Obsessive Beliefs Questionnaire; BDI = the Beck Depression Inventory; SAI = State Anxiety Inventory.

Overestimation of threat was measured with the Threat Estimation subscale of the Obsessive Beliefs Questionnaire (OBQ-44). The OBQ-44 was developed by the Obsessive Compulsive Cognitions Working Group to measure beliefs that are thought to be related to the maintenance of OCD (Obsessive Compulsive Cognitions Working Group (OCCWG), 2001). It consists of six belief domains: Responsibility, Threat Estimation, Perfectionism, Intolerance of Uncertainty, Importance of Thoughts, and Control of Thoughts. The OBQ-44 has demonstrated convergent validity with measures OCD symptoms and OBQ-44 total scores have been shown to be significantly higher in OCD patients than community controls, student controls, and anxious controls (OCCWG, 2005). A factor analysis suggested these items could be grouped into three belief dimensions: Responsibility/Threat Estimation, Perfectionism/Intolerance of Uncertainty, Importance/Control of Thoughts (OCCWG, 2005). The use of the Threat Estimation items as a scale of overestimation of threat is supported by a later factor analysis of the OBQ-44 that suggests that the Threat Estimation subscale is a unique factor (Meyers, Fischer, & Wells, 2008). The high Threat Estimation group (Threat Estimation ≥ 21) consisted of 32 individuals (17 females, 15 males) while the low Threat Estimation group (Threat Estimation ≤ 20) consisted of 27 individuals (21 females, 6 males). Table 2.2 shows the demographics for the high and low Threat Estimation groups. No between-group differences in age were apparent.

All participants had normal or corrected-to-normal vision and hearing and no history of a major neurological condition. Participants were excluded if they were currently using psychoactive medications. This study was approved by the University of

Minnesota Institutional Review Board and informed consent was obtained from all participants. Participants received extra credit in an introductory course for their participation.

Table 2.2

Group characteristics for the high and low Threat Estimation groups

	Low Threat Estimation	**High Threat Estimation**
Sample size (females, males)	27 (21, 6)	32 (17, 15)
Age in years	21.19 (4.10)	20.78 (3.06)
Threat Estimation from the OBQ-44	14.56* (3.63)	26.88* (5.27)
OCI-R	8.83* (9.26)	24.44* (14.38)
BDI	6.27* (6.78)	15.11* (9.99)
SAI	33.75* (6.61)	43.38* (12.29)

Note. Group means and (standard deviations) are reported; Significant group differences ($p < .05$) are denoted with an asterisk. OCI-R = the Obsessive-Compulsive Inventory-Revised; OBQ-44 = Obsessive Beliefs Questionnaire; BDI = the Beck Depression Inventory; SAI = State Anxiety Inventory.

Physiological Apparatus

Fear-potentiated startle was recorded using a commercial system (PsychLab psychophysiologic recording system, Precision Instruments), which also administered mild electric shocks to the non-dominant wrist of subjects. The shocks were delivered

through two-disk electrodes placed on the participants' non-dominant wrist. Participants received between 1-3 sample shocks prior to the start of the experiment, which they rated in terms of painfulness on a scale from 0 (no pain) to 5 (extremely painful). The shock level was adjusted based on our assessment of their tolerance. The shocks had an intensity between 3 to 5 milliamperes and duration of 100 ms. Fear-potentiated startle (startle blink or electromyography) was measured using two 6mm silver-chloride electrodes positioned under the left eye (sampling rate=1000 Hz; bandwidth=30–500 Hz). A ground electrode was placed on the participant's non-dominant forearm. Startle probes consisted of 40ms, 102 dBA bursts of white noise with a near instantaneous rise time presented binaurally through headphones.

Conditioned Generalization Paradigm

The conditioned generalization paradigm used in this study has been described in detail elsewhere (Lissek et al., 2008; 2010). This paradigm involves the presentation of 10 rings of gradually increasing size, where the largest and smallest rings serve as the conditioned danger cue (paired with an unconditioned stimulus; CS+) or the conditioned safety cue (not paired with an unconditioned stimulus; CS-). An electric shock (3–5 mA) delivered to the participant's non-dominant wrist was used as the unconditioned stimulus. The generalization stimuli consisted of eight intermediately sized rings that form a continuum of similarity between the CS+ and CS- (see Figure 2.1). Rings were presented for eight seconds on a computer monitor using Presentation software. Prior to the start of the study, participants underwent habituation to nine startle probes.

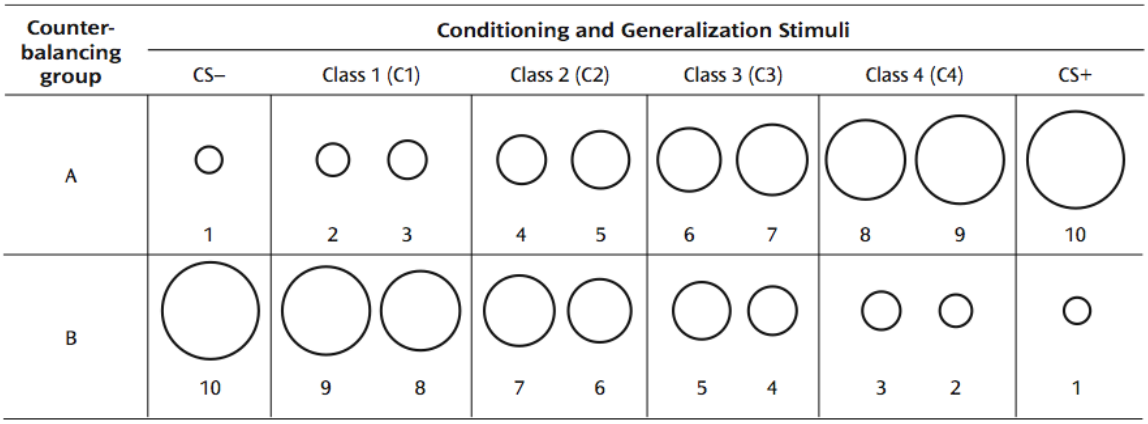

Figure 2.1: Conditioning and Generalization Stimuli. Groups were counterbalancing so that for half of the participants, the largest ring was the conditioned danger cue and the smallest ring was the safety cue (counterbalancing group A) and for the other half, the stimuli were reversed (counterbalancing group B). The eight intermediate ring sizes were grouped into four classes (C1, C2, C3, and C4) to avoid an excessive number of trials while maintaining a gradual continuum of ring sizes (see Lissek et al., 2010).

The conditioned generalization paradigm consists of three phases: preacquisition (presentation of the CS- and CS+ stimuli without shocks), acquisition (fear conditioning with the CS- and CS+), and generalization (presentation of the CS-, CS+, and the eight generalization stimuli). The trial types and frequencies for each phase are listed in table 2.3. During each phase, half of the trials were followed by startle probes that occurred 4 or 5 seconds after onset of the conditioned or generalization stimulus. A balanced number of startle probes were presented during inter-trial intervals. Startle probes were separated by 18-25 second time intervals throughout the study.

During stimulus trials and inter-trial intervals without startle probes, behavioral ratings (perceived risk for shock) and response times were collected. Participants were shown the question “Level of risk?” presented above the stimulus 1 to 2 seconds after trial onset, which cued participants to rate their perceived likelihood of receiving a shock

on a 3-point scale (1=no risk, 2=moderate risk, and 3=high risk). Participants were instructed to respond as quickly as possible with their dominant hand using a subject response box. Additionally, retrospective self-reported levels of anxiety evoked by conditioned danger and conditioned safety cues were collected using 10-point Likert scales (1=none, 5=some, 10=a lot) following the acquisition and generalization phases.

Table 2.3

Trial types and frequencies during preacquisition, acquisition, and generalization test

	Conditioning and Generalization Stimuli							
						CS+		
Phase	CS-	C1	C2	C3	C4	Coterminated with UCS	Not Coterminated with UCS	ITI
Preacquisition	6	-	-	-	-	0	6	6
Acquisition	12	-	-	-	-	9	3	12
Generalization test	12	12	12	12	12	6	6	12

Note. CS−=conditioned safety cue; CS+=conditioned danger cue; C1, C2, C3, and C4=generalization stimulus classes 1, 2, 3, and 4; UCS=unconditioned stimulus; ITI=inter-trial intervals. During the generalization test, the CS+ continued to be reinforced with shock to avoid extinction of the conditioned response during the generalization sequence.

Mood and Anxiety Questionnaires

Prior to the physiological recording session, participants completed a battery of questionnaires online including the Obsessive Beliefs Questionnaire (OBQ-44; OCCWG, 2005), the Obsessive-Compulsive Inventory-Revised (OCI-R; Foa et al., 2002), the State Anxiety Inventory (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983), and the Beck Depression Inventory (BDI; Beck & Steer, 1987) as well as a demographics questionnaire.

Data Analysis

Startle electromyography (EMG) raw data was rectified and smoothed using a 20 ms moving window average. The onset latency window for the startle EMG response was 20–100 ms. Peak EMG magnitude was determined by taking the peak value between 21 and 120 ms following stimulus onset (startle probe) and subtracting the average baseline EMG level 50 ms prior to the stimulus onset. For each trial, a zero response was scored if no peak magnitude was detectable (i.e., EMG magnitude less than 1 microvolt). Criteria for trial rejection included unstable baseline EMG activity or peak EMG magnitudes occurring within 20 ms of startle probe onset. The percentage of trials that were rejected based on these criteria was similar in the high and low OCI-R groups as well as the high and low Threat Estimation groups.

EMG magnitudes were standardized using with-in subject T scores. EMG magnitudes during acquisition phase were analyzed with a 2×2 repeated measures analysis of variance (ANOVA): group (high and low OCI-R) by stimulus (danger cue and safety cue). EMG magnitudes during the generalization phase were analyzed with a 2×6 repeated measures analysis of variance (ANOVA): group (high and low OCI-R) by stimulus type (safety cue, Class 1, Class 2, Class 3, Class 4, and danger cue). Four individuals were excluded from startle EMG analyses because of equipment problems. ANOVAs were computed using Wilks's lambda and were followed, when necessary, by either trend analyses or paired-samples t tests. Geisser-Greenhouse corrections were used when there were violations of the sphericity assumption. The shape of generalization gradients were tested using quadratic trend analyses based on a priori predictions that the

high OC and high Threat Estimation groups would show a more linear gradient of EMG magnitudes which would reflect greater generalization in these groups. Risk ratings and startle EMG magnitudes were transformed into a measure of deviation from linearity (Mean(CS+ and CS-) - Mean (Classes 1, 2, 3, and 4)) in order to obtain a single continuous measure that characterizes the generalization slope, which can then be correlated with other variables of interest such as symptom questionnaires. Alpha was set at 0.05 and was corrected using Hochberg's adjustment for multiple tests where appropriate (Hochberg, 1988).

Results

Pre-Acquisition

During pre-acquisition, no main effects of stimulus type or stimulus type-by-group interactions were found for startle EMG, suggesting that prior to conditioning, there were no group differences in startle reactions for the danger and safety cues in either the high and low OCI-R groups or the high and low Threat Estimation groups (*p*-values $\geq .14$).

Acquisition

Startle EMG. Means and standard deviations are displayed in table 2.4 for the high and low OCI-R groups and table 2.5 the high and low Threat Estimation groups. A 2x2 group-by-stimulus ANOVA revealed significant main effects for stimulus type in the high and low OCI-R groups ($F(1, 54)=40.77$, $p < .001$) and in the high and low Threat Estimation groups ($F(1, 54)=39.22$, $p < .001$). Fear potentiated startle was greater for the danger cue than the safety cue in both the high OCI-R ($t(26)=4.31$, $p < .001$) and low

OCI-R ($t(28)=4.84$, $p < .001$) groups as well as the high Threat Estimation ($t(30)=4.31$, $p < .001$) and low Threat Estimation ($t(24)=5.22$, $p < .001$) groups, showing that all groups were able to condition to the danger cue. There were no significant group-by-stimulus interactions during acquisition, suggesting that the strength of the fear potentiated startle did not differ across groups (p-values $\geq .48$).

Retrospective anxiety. Conditioning to the danger cue was also apparent using self-reported measures of anxiety administered post-acquisition. Higher levels of anxiety to the conditioned danger cue compared to the conditioned safety cue were reported in both the high OCI-R group ($t(27)= 7.96$, $p < .001$, danger cue: Mean= 8.04, SD=1.90, safety cue: Mean= 3.68, SD= 2.20) and the low OCI-R group ($t(30)= 10.83$, $p < .001$, danger cue: Mean= 7.26, SD= 1.95, safety cue: Mean= 2.61, SD= 1.33). Higher levels of anxiety to the conditioned danger cue were also reported in both the high Threat Estimation group ($t(31)= 9.65$, $p < .001$, danger cue: Mean= 8.06, SD= 1.78, safety cue: Mean= 3.50, SD= 2.11) and the low Threat Estimation group ($t(26)= 8.86$, $p < .001$, danger cue: Mean= 7.11, SD= 2.04, safety cue: Mean= 2.67, SD= 1.41) . No stimulus type-by-group interactions were found (p-values $\geq .68$); however, main effects for group were found (OCI-R: $F(1, 57)= 7.28$, $p = .009$; Threat Estimation: $F(1, 57)= 6.74$, $p = .012$) indicating that the high OCI-R group and the high Threat Estimation group reported greater anxiety to both the danger and safety cues than the low OCI-R or low Threat Estimation groups.

Table 2.4

Acquisition data for standardized startle EMG across conditioned danger cues (CS+), conditioned safety cues (CS–), and inter-trial intervals for the high and low OCI-R groups

	Startle EMG[a]			
Stimulus	High OCI-R		Low OCI-R	
	Mean	SD	Mean	SD
CS+	54.75	4.51	55.60	4.28
CS-	49.20	4.25	51.16	3.18
ITI	51.22	4.49	49.42	3.06

[a]Raw startle EMG was standardized with the use of within-subject T score transformations ([([EMG single trial – EMGmean]/SD)*10] + 50). OCI-R = Obsessive-compulsive Inventory-Revised; CS+ = conditioned danger cue; CS- = conditioned safety cue; ITI = inter-trial interval.

Table 2.5

Acquisition data for standardized startle EMG across conditioned danger cues (CS+), conditioned safety cues (CS–), and inter-trial intervals for the high and low Threat Estimation groups as measured by the OBQ-44

	Startle EMG[a]			
Stimulus	High Threat Estimation		Low Threat Estimation	
	Mean	SD	Mean	SD
CS+	55.36	4.60	54.99	4.16
CS-	50.07	4.65	50.39	2.55
ITI	51.04	4.10	49.36	3.46

[a] Raw startle EMG was standardized with the use of within-subject T score transformations ([([EMG single trial – EMGmean]/SD)*10] + 50). OCI-R = Obsessive-compulsive Inventory-Revised; CS+ = conditioned danger cue; CS- = conditioned safety cue; ITI = inter-trial interval.

Generalization Test

Startle EMG. A 2x2 stimulus type-by-group repeated measures ANOVA revealed significant main effects for stimulus type (safety vs. danger cue) in the high and low OCI-R groups ($F(1, 53)=31.85$, $p < .001$) and in the high and low Threat Estimation groups ($F(1, 53)=33.66$, $p < .001$). Fear potentiated startle was greater for the danger cue than the safety cue in both the high OCI-R ($t(25)=3.63$, $p = .001$) and low OCI-R ($t(28)=4.38$, $p < .001$) groups as well as the high Threat Estimation ($t(29)=3.39$, $p = .002$) and low Threat Estimation ($t(24)=4.86$, $p < .001$) groups, which suggests that conditioned fear to the danger cue persisted during the generalization test. No significant stimulus type-by-group interactions were found (p-values $\geq .29$). No gender differences were found in startle EMG magnitudes for any of the stimuli: safety cue, Classes 1-4, or danger cue (p-values $\geq .40$).

A 2x6 repeated measures ANOVA revealed main effects of stimulus type for the high and low OCI-R groups ($F(5,49)=10.44$, $p < .001$) and the high and low Threat Estimation groups ($F(5,49)=10.91$, $p < .001$). Specifically, the generalization gradients were characterized by downward slopes in startle magnitude as the stimulus becomes less similar to the conditioned danger cue (see Figures 2.2 and 2.3). Both the high and low OCI-R groups showed significant quadratic slopes ($F(1,53)=23.96$, $p < .001$) with no significant stimulus type-by-group interaction ($p = .69$). The high and low Threat Estimation groups also showed significant quadratic slopes ($F(1,53)=25.85$, $p < .001$) and there was an interaction between Threat Estimation group and response slopes from the danger cue to stimulus Class 4 ($F(1,53)=6.30$, $p = .015$). The low Threat Estimation

group showed a steep decline in fear potentiated startle between the danger cue and the next class of stimuli (Class 4) while the high Threat Estimation group shows a less steep decline in fear potentiated startle (see Figure 2.3).

Planned comparisons between the conditioned safety cue and the four classes of generalization stimuli, as well as the danger cue, were conducted to determine the point at which startle magnitude was significantly different from the safety cue, indicating that discrimination learning (the opposite of generalization) has occurred. These five contrasts were corrected for multiple comparisons using Hochberg's adjustment. Using a criterion of $p = .02$, the results show that startle EMG magnitudes were significantly larger for the danger cue than for the safety cue in both the high OCI-R group ($p = .001$) and low OCI-R group ($p < .001$), suggesting that both groups conditioned to the danger cue to the same degree. Similarly, startle EMG magnitudes were also larger for the danger cue than for the safety cue in both the high Threat Estimation group ($p = .002$) and the low Threat Estimation group ($p < .001$). Startle EMG magnitudes did not significantly differ between the safety cue and the other classes of generalization stimuli in either the high or low OCI-R groups.

In contrast, startle magnitudes were significantly larger for the Class 4 generalization stimuli than for the safety cue in the high Threat Estimation group ($t(29)=3.14$, $p = .004$) but were not larger relative to the safety cue in the low Threat Estimation group ($t(24)=1.27$, $p = .22$). The low Threat Estimation group was able to suppress the fear response to the next class of stimuli that resembled the danger cue while the high Threat Estimation group showed similar levels of fear to both the danger cue and

the next class of similarly sized stimuli, suggesting overgeneralization of the conditioned fear response.

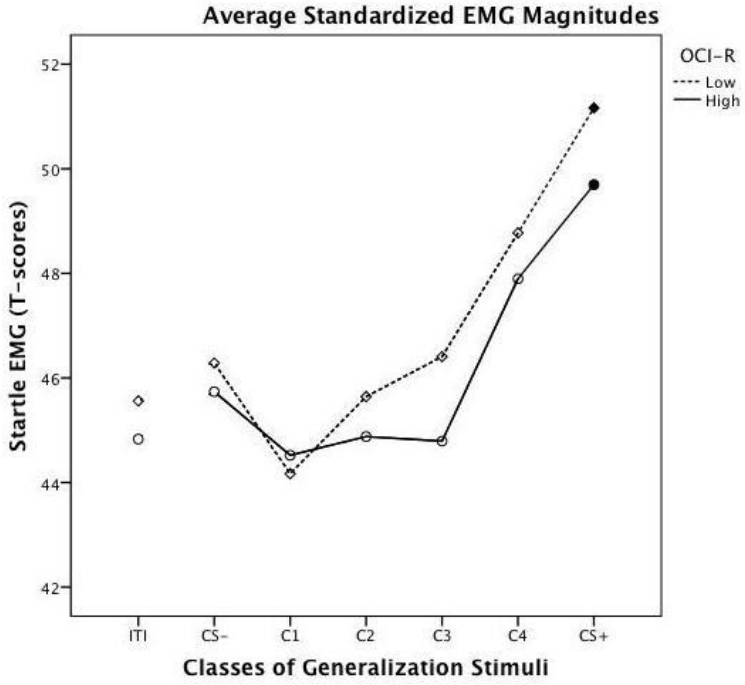

Figure 2.2. Average standardized startle EMG magnitudes during the generalization test by group (high and low Obsessive-compulsive Inventory-Revised), for the inter-trial interval (ITI), safety cue (CS–), four classes of generalization stimuli (C1, C2, C3, C4), and danger cue (CS+). Black dots indicate that the startle EMG magnitudes were significantly larger for the danger cue than for the safety cue.

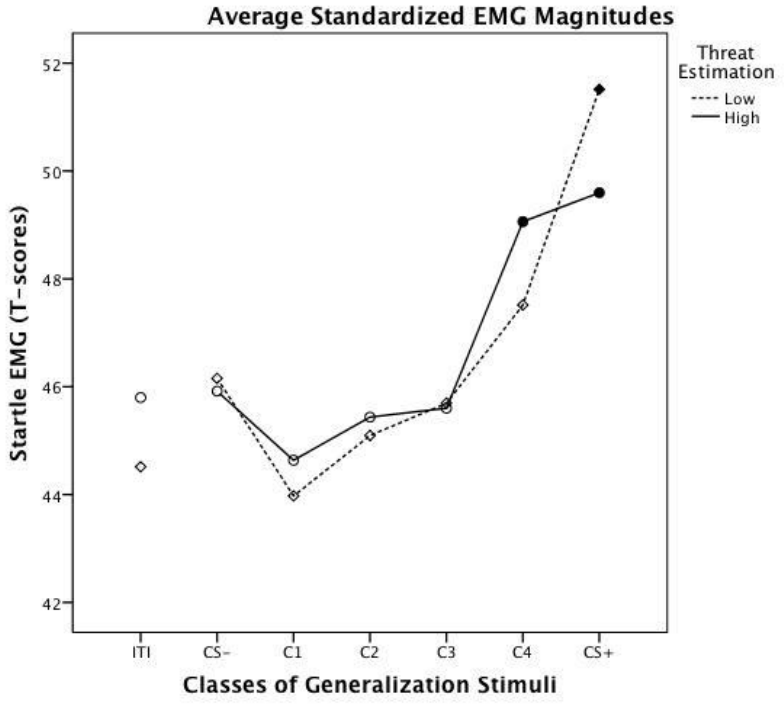

Figure 2.3. Average standardized startle EMG magnitudes during the generalization test by group (high and low Threat Estimation), for the inter-trial interval (ITI), safety cue (CS–), four classes of generalization stimuli (C1, C2, C3, C4), and danger cue (CS+). Black dots indicate that the startle EMG magnitudes were significantly larger for the danger cue and class 4 (C4) than for the safety cue in the high Threat Estimation group, but were only larger for the danger cue in the low Threat Estimation group.

Risk ratings. During generalization, no main effects for group or stimulus type-by-group interactions were found for risk ratings in either the high and low OCI-R groups or the high and low Threat Estimation groups (*p*-values ≥ .068). There was a significant main effect for stimulus type in the high and low OCI-R groups and the high and low Threat Estimation groups (*p*-values < .001). The high and low OCI-R groups and the high and low Threat Estimation groups were characterized by significant quadratic declines in risk ratings as the stimuli decreased in similarity to the conditioned danger cue (*p*-values ≤ .005). Risk ratings and startle EMG magnitudes were transformed into a measure of

deviation from linearity (Mean(CS+ and CS-) - Mean (Classes 1, 2, 3, and 4)). Using these measures, risk ratings were positively correlated with startle EMG magnitudes ($r(53) = .40$, $p = .003$), suggesting that higher risk ratings are associated with more linear (less steep) declines in startle EMG as the stimuli become less similar to the conditioned danger cue.

Reaction times. In the high and low OCI-R groups and the high and low Threat Estimation groups, no stimulus type-by-group interactions were found for reaction times (p-values $\geq .58$). No main effect for group was found for the OCI-R groups ($p = .37$). Although the main effect for group did not reach significance in the Threat Estimation groups either ($p = .055$), comparisons of the means suggests slower response times in the high Threat Estimation group for all stimuli (safety cue, Classes 1-4, and danger cue). For the reactions times, a significant main effect for stimulus type was found (p-values = .001). All groups were characterized by an inverted U shape, suggesting slower reaction times to the generalization stimuli (Classes 2, 3, and 4), consistent with previous research showing slower responding for stimuli with less certain threat information than the conditioned danger and safety cues (Lissek et al., 2010).

Symptom questionnaires. The State Anxiety Inventory was positively correlated with Threat Estimation ($r(57) = .52$, $p < .001$) as was the BDI ($r(56) = .61$, $p < .001$). Correlations between these measures and risk ratings or startle magnitudes were not significant (p-values $\geq .37$).

Retrospective anxiety. Self-reported levels of anxiety also show that conditioned anxiety for the danger cue persisted during the generalization test. Higher levels of

anxiety to the conditioned danger cue were reported in both the high OCI-R group ($t(27)= 9.35$, $p < .001$, danger cue: Mean= 7.86, SD=2.55, safety cue: Mean= 2.32, SD= 1.52) and the low OCI-R group ($t(30)= 15.09$, $p < .001$, danger cue: Mean= 6.77, SD= 2.03, safety cue: Mean= 1.42, SD= .67). There was a main effect for group suggesting higher self-reported levels of anxiety to both the danger and safety cues in the high OCI-R group but not the low OCIR group ($F(1,57) = 9.01$, $p = .004$). No stimulus type-by-group interaction was found, suggesting that the pattern of anxiety levels to the danger and safety cues did not differ between the groups ($p = .79$). Higher levels of anxiety to the conditioned danger cue were also reported in both the high Threat Estimation group ($t(31)= 11.02$, $p < .001$, danger cue: Mean= 7.63, SD= 2.45, safety cue: Mean= 2.09, SD= 1.47) and the low Threat Estimation group ($t(26)= 12.30$, $p < .001$, danger cue: Mean= 6.89, SD= 2.17, safety cue: Mean= 1.56, SD= .80). No significant main effect for group or stimulus type-by-group interaction was found (p-values $\geq .07$).

Discussion

This study represents the first attempt to study the generalization of conditioned fear in individuals with obsessive-compulsive traits. The results of this study suggest that individuals with high levels of Threat Estimation as measured by the Obsessive Beliefs Questionnaire (OBQ-44) display overgeneralization of fear responses to a greater range of stimuli resembling the danger cue than those with low levels of Threat Estimation. In particular, the high Threat Estimation group showed greater fear-potentiated startle to ring sizes up to two units of dissimilarity from the danger cue while the low Threat Estimation group did not generalize the conditioned fear response beyond the danger cue.

This suggests that the high Threat Estimation group may be characterized by lower thresholds of threat reactivity, which results in greater fear responses to stimuli that resemble the danger cue.

Similar to the animal literature and to previous research on the generalization of conditioned fear in humans (Lissek et al., 2009; 2010), this study found that the generalization gradients were characterized by quadratic declines in conditioned fear as the presented stimuli became less similar to the danger cue. Unlike the Lissek et al. (2010) study, the present study was not able to replicate the more gradual, linear declines in conditioned fear responding that the authors found in individuals with panic disorder. This divergence in findings may be due to the type of population used in the present study. A limitation to the current study is use of a non-clinical population of individuals with obsessive-compulsive traits rather than patients with OCD. Linear declines in conditioned fear responding may be more apparent when using clinical patients with OCD. It is also possible that linear declines in fear responding are more characteristic of particular anxiety disorders, such as panic disorder and GAD, rather than obsessive-compulsive disorder.

No differences in generalization were found when comparing individuals with high and low levels of overall obsessive-compulsive symptoms as measured by the Obsessive-compulsive Inventory-Revised (OCI-R). Generalization effects in this study were restricted to comparisons between the high and low Threat Estimation groups from the OBQ-44, suggesting that only a subset of individuals with obsessive-compulsive traits show overgeneralization. The lack of group differences in the high and low obsessive-

compulsive groups may also be due to the lack of disorder-relevant conditioned stimuli used in this study's experimental paradigm. Lissek, Pine, and Grillon (2006) have noted that paradigms that use threat of shock as the unconditioned stimulus may not be as pertinent to anxiety disorders where threat of physical harm is not a key feature. For example, fear of physical harm is characteristic of posttraumatic stress disorder but is less relevant to obsessive-compulsive disorder. Experimental paradigms that use unconditioned stimuli such as contamination may be more successful at finding group differences in fear responses in obsessive-compulsive populations.

Furthermore, the finding of greater generalization in the high Threat Estimation group compared to the low Threat Estimation group and no differences in the high and low obsessive-compulsive groups may be due to overestimation of threat being non-specific to OCD. Research using the OBQ-44 subscales has suggested that beliefs about the importance of thoughts and control of thoughts reliably differentiate OCD patients from other non-obsessive anxiety patients whereas other dimensions such as threat estimation, responsibility, perfectionism, and intolerance of uncertainty may be less specific to OCD (Anholt et al., 2006; Clark, Purdon, & Wang, 2003; Sica et al., 2004; Tolin, Worhunsky, & Maltby, 2006). In particular, overestimation of threat is common across many anxiety disorders including GAD, OCD, PTSD, and panic disorder. Therefore, we would expect to find overgeneralization in other disorders characterized by high levels of threat reactivity and this has been supported in research on panic and GAD patients and is currently being investigated by the authors in PTSD patients. The current study's finding of overgeneralization in those high in Threat Estimation coupled with

previous research showing greater generalization in anxiety disorders characterized by threat reactivity supports the notion of overestimation of threat possibly being one important process underlying the overgeneralization of fear.

In conclusion, the results of this study suggest that overestimation of threat may be an important precursor to the generalization of conditioned fear. In particular, group differences in conditioned generalization were only found in the high and low Threat Estimation groups as measured by the OBQ-44 and not in the high and low obsessive-compulsive groups as measured by the OCI-R. This suggests that not all individuals with OCD traits are characterized by overgeneralization of fear; specifically, individuals who overestimate threat appear to be at risk for overgeneralizing their fear. The overgeneralization of conditioned fear remains an important but understudied process in the research on anxiety disorders. Future studies in this area would benefit from the use disorder-specific unconditioned stimuli and from the replication of the current study's results in a population of OCD patients.

Chapter 3: The Generalization of Conditioned Fear in PTSD

Posttraumatic stress disorder (PTSD) is characterized by feelings of intense fear, helplessness, or horror, increased arousal, and avoidance following a traumatic event (PTSD; American Psychiatric Association, 2000). Etiological explanations of PTSD posit fear conditioning as a primary mechanism by which symptoms of PTSD are developed and maintained. Conditioned fear results from the pairing of an unconditioned aversive stimulus (US) with a neutral stimulus (conditioned stimulus or CS), leading the neutral stimulus to elicit anxiety associated with the anticipation of the aversive stimulus (conditioned response or CR). In a fear conditioning account of PTSD, individuals with a vulnerability to developing this disorder (e.g., genetic predisposition, abnormalities in fear conditioning) who experience a sufficiently aversive event will show a fear conditioned response that is maladaptive (e.g., excessively prolonged reactivity, generalization to benign stimuli).

Symptoms of PTSD such as re-experiencing the event and avoidance of stimuli associated with the trauma are thought to be directly related to this fear conditioning process. There is a straightforward association between experiencing a traumatic event and subsequent excessive threat reactivity to trauma-relevant stimuli in PTSD patients which suggests that dysregulation of fear conditioning processes may be important in the development of psychopathology to trauma. Fear conditioning accounts of PTSD have focused on abnormalities in the acquisition, inhibition, extinction, associative learning, avoidance, or overgeneralization of conditioned fear (see Lissek & Grillon, 2012 for an overview). Overgeneralization refers to a propensity to generalize conditioned fear to

stimuli that resemble the original threat (Lissek et al., 2008; 2010). For example, a soldier who is conditioned to fear road side objects following an explosion while in a war zone may generalize that fear to benign road side objects upon returning home despite safety signals in the environment (familiar, non-war related surroundings) that should inhibit threat reactivity. A lower threshold for threat reactivity is thought to predispose the individual to overgeneralize fear to benign objects/situations that resemble the conditioned danger cue. In individuals with a lower threshold for threat reactivity, less threat information is needed to activate a fear response which will lead to reactivity to a larger range of stimuli resembling the danger cue; hence, the overgeneralization of fear.

Despite the importance of conditioned fear generalization in etiological accounts of PTSD, there is a paucity of research on the overgeneralization of conditioned fear in PTSD. The finding of overgeneralization of conditioned fear to stimuli that are perceptually similar to a conditioned danger cue is a robust finding in anxiety patients (Lissek et al., 2005). Studies have found enhanced fear responses to safety cues in PTSD patients using a discrimination learning paradigm (Grillon & Morgan, 1999; Jovanovic et al., 2010; Orr et al., 2000; Peri et al., 2000; for opposing results see Grillon et al., 1998; Morgan et al., 1995). Discrimination fear conditioning involves the presentation of a conditioned danger cue (CS+) that is paired with an aversive stimulus and a safety cue (CS-) that is not paired with an aversive stimulus. Because CS- typically shares similar features to the CS+ (both may be similar in size, shape, or spatial location), the finding of enhanced fear responses to CS- suggests that PTSD patients may be generalizing their fear to stimuli that resemble CS+.

An experimental paradigm designed specifically to test the generalization of conditioned fear was developed by Lissek et al. (2008). The authors adapted the generalization paradigms used in animal studies and applied a similar experimental design to human research (for reviews of generalization paradigms, see Honig & Urcuioli, 1981; Kalish, 1969; Mackintosh, 1974). This paradigm involves the presentation of rings of graded size where the smallest and largest rings serve as either CS+ (paired with an electric shock) or CS- (no shock) and where eight rings of intermediate size serve as generalization stimuli that form a continuum-of-similarity between the danger and safety cue. Generalization is captured by the steepness of the downward gradient, or slope, in fear responding as the target stimulus differentiates from the danger cue, with less steep downward slopes indicating stronger generalization.

Research using this paradigm has demonstrated that healthy participants show steep quadratic declines in fear responses to the generalization stimuli, suggesting relatively rapid declines in fear responding as the stimulus becomes less similar to CS+ (Lissek et al., 2008). Conversely, panic disorder and generalized anxiety disorder patients demonstrated linear, more gradual declines in fear responses to the generalization stimuli, suggesting heightened fear responses to stimuli that resemble CS+ (Lissek et al., 2010, Lissek et al., under review). These results implicate overgeneralization of conditioned fear as an important correlate of clinical anxiety. Such overgeneralization may be particularly relevant to PTSD for which over-reactivity to stimuli resembling the traumatic event is a central feature; however, there are currently no published studies specifically aimed at assessing generalization of conditioned fear in PTSD patients.

Furthermore, little is known about the potential abnormalities in brain mechanisms associated with fear generalization in PTSD. Neural structures that are thought to be important for fear conditioning include the amygdala, the insula, the bed nucleus of the stria terminalis (BNST), the ventral medial prefrontal cortex (vmPFC) and the hippocampus (Davis & Shi, 1999; Jovanovic & Ressler, 2010; Kim & Fanselow, 1992; Lissek et al., 2013; Phillips & LeDoux, 1992). Lissek et al. (2013) propose a neural model of conditioned fear generalization in which the sensory cortex and the hippocampus assist in pattern-matching between the encountered stimulus and previous learned conditioned danger cues. This sensory cortex/hippocampal processing network is thought to activate brain areas associated with fear excitation (e.g., amygdala, insula), in the instance of pattern matches, and fear inhibition (e.g., vmPFC), in the instance of pattern mismatches.

Studies using fear conditioning paradigms support the finding of abnormalities in these brain areas in PTSD patients relative to controls, including increased activation of the amygdala and decreased activation in the anterior cingulate during extinction (Bremner et al., 2005), increased amygdala activation during extinction learning but decreased hippocampus and vmPFC activation during extinction recall (Milad et al., 2009), and decreased vmPFC activation during threat of shock (Tuescher et al., 2011). Because the vmPFC is thought to inhibit activation of the limbic system, including the amygdala (Grace & Rosencrantz, 2002; Morgan, Romanski, & LeDoux, 1993; Phelps et al., 2004), the finding of excessive amygdala activation and decreased vmPFC activation

suggests that PTSD patients may be associated with deficits in both excitatory and inhibitory neural circuits.

Research specific to the generalization of conditioned fear implicates a network of brain areas subserving conditioned fear generalization including bilateral insula, dorsomedial prefrontal cortex (dmPFC), bilateral inferior parietal lobule, striatum, thalamus, subgenual cingulate, anterior cingulate cortex, caudate nucleus, bilateral hippocampus, ventromedial prefrontal cortex (vmPFC), and precuneus (Dunsmoor et al., 2011, 2012; Greenberg et al., 2013a, 2013b; Lissek et al., 2013). However, Dunsmoor and colleagues use an intensity-based generalization paradigm that involves both the perceptual similarity of the GSs to the CS+ and the emotional intensity of GSs (i.e., low, medium, or high levels of fearful facial expressions), making the two effects difficult to disentangle. Furthermore, Greenberg and colleagues employed an instructed threat generalization paradigm in which participants are informed that the GSs will never be paired with shock. Instructed threat involves higher-level cognitive reasoning, while the paradigm used by Lissek et al. (2013) is based on classical conditioning and may be more relevant to translational research between animals and humans.

The generalization paradigm proposed by Lissek and colleagues provides an optimal method for testing classically conditioned fear generalization effects in PTSD that are not based on secondary emotional valence or highly cognitive instructed threat. Using this paradigm in healthy participants, Lissek et al. (2013) found activations in the insula, dorsomedial prefrontal cortex, and bilateral inferior parietal lobule were strongest to the learned danger cue and decrease in a curve-linear manner as the presented stimulus

diverged in similarity from the danger cue. Inversely, activations in the hippocampus, vmPFC, and precuneus were strongest to the conditioned safety cue and decreased gradually as the presented stimulus becomes more similar to the conditioned danger cue (Lissek et al., 2013). However, there are currently no studies using this paradigm to explore group differences in the neural mechanisms subserving conditioned fear generalization in PTSD patients.

The aim of the current study is to identify differences in the brain mechanisms associated with conditioned fear generalization between PTSD patients and controls. More specifically, this study aims to: 1) determine the degree to which veterans with PTSD generalize conditioned fear when compared to non-trauma controls, trauma controls, and individuals with sub-threshold PTSD, and 2) identify brain processes associated with the generalization of conditioned fear in PTSD. This study hypothesizes that veterans with PTSD will show stronger conditioned generalization than the control groups as evidenced by less steep, downward generalization slopes in behavioral indices of perceived threat, skin conductance responses, and fear-related brain areas (insula, dorsal medial prefrontal cortex, amygdala), as well as less steep upward slopes in brain areas associated with fear inhibition (ventral medial prefrontal cortex, hippocampus, precuneus).

Methods

Participants

Participants included 79 male adults with a mean age of 33.46 (SD=9.62) who were recruited from the Minneapolis Veterans Affairs Medical Center, the University of

Minnesota, and local community colleges. All participant received reimbursement for their time. This study was approved by both the Minneapolis VA Institutional Review Board and the University of Minnesota Institutional Review Board and informed consent was obtained from all participants prior to testing. This study consisted of four groups: posttraumatic stress disorder (PTSD), sub-threshold PTSD, trauma controls, and non-trauma controls (see table 3.1 for group demographics). The groups did not differ in terms education levels (p = .45) and group differences in age did not reach significance (p = .11). When age is entered as a covariate, the pattern of results remains the same.

A diagnosis of PTSD was made using the Clinician Administered PTSD Scale for the DSM-IV (CAPS; Blake et al., 1990, Blake et al., 1995). A sub-threshold PTSD group was comprised of individuals with a trauma history but no current diagnosis of PTSD and a CAPS score between 20 and 39 based on the CAPS severity ratings suggested by Weathers, Keane, and Davidson (2001): 0-19 - asymptomatic/few symptoms, 20-39 - sub-threshold PTSD, 40-59 - Moderate PTSD/threshold, 60-79 - severe PTSD, ≥80 - Extreme PTSD. The trauma control group consisted of individuals with a history of trauma but no past or present diagnosis of PTSD. The non-trauma control group was comprised of individuals without trauma history. Exclusion criteria included: 1) any medical condition, implant, or device that was not safe for the MRI environment; 2) current Axis I psychiatric disorder in the trauma controls or non-trauma controls as determined by the Structured Clinical Interview for DSM-IV (SCID-I; First et al., 2001); 3) evidence of significant suicidal ideation or behavior; 4) individuals who currently (or during the last six months) met criteria for substance abuse or dependence; 5) any major

medical condition that interfered with the objectives of the study (e.g., history of organic mental disorders, seizure, or mental retardation); and 6) current use of illicit drugs.

Table 3.1: Group demographics and symptoms questionnaires.

	PTSD	**Sub-threshold PTSD**	**Trauma Controls**	**Non-trauma Controls**
Sample size	26	19	17	17
Age in years	33.73 (9.12)	36.26 (9.58)	34.59 (9.27)	28.76 (9.91)
CAPS	61.42* (16.20)	31.11* (7.73)	13.65* (6.77)	6.12* (6.86)
PCL	54.12* (12.55)	43.37* (10.60)	33.12* (10.12)	27.00* (8.49)
ASI	26.85* (13.37)	20.63 (11.26)	15.18* (7.54)	11.06* (7.00)
State anxiety	47.62* (12.19)	41.42 (12.15)	32.18* (10.21)	31.00* (7.05)
Trait anxiety	51.31* (10.35)	45.00 (12.82)	36.65* (11.19)	36.29* (6.82)
BDI	18.81* (7.67)	12.95* (8.24)	9.24* (7.47)	5.35* (3.81)
FPS	69.04 (20.25)	63.89 (14.74)	69.47 (19.80)	67.65 (18.82)

Note. Group means and (standard deviations) are reported; Significant group differences ($p < .05$) are denoted with an asterisk. PTSD = Posttraumatic Stress Disorder; CAPS = Clinician Administered PTSD Scale; PCL = PTSD Checklist; ASI = Anxiety Sensitivity Index; BDI = the Beck Depression Inventory; FPS = Fear of Pain Scale.

Generalization Task

The generalization task is based on the generalization paradigm developed by Lissek et al. (2010) and has been modified to incorporate the design parameters of fMRI retinotopic mapping studies (e.g., Murray, Boyaci, & Kersten 2006; Schwartz, 2005). The

stimuli consisted of 5 checkerboard counterphase-flickering (10 Hz) rings of graded size and a checkerboard "V" (see Figure 3.1). Counter-phase flickering checkerboards were used to maximally activate the calcarine sulcus (Murray et al., 2006) in order to retinotopically map representations of CSs and GSs in sensory cortex (retinotopic data not reported in this paper).

In the current paradigm, there was one ring-shaped danger cue (CS+) and two safety cues (CS-): one ring-shaped (referred to as the oCS-) and one "V" shaped (referred to as the vCS-). For half of participants, the smallest ring was designated CS+ and paired with an electric shock and the largest ring was oCS- and not paired with shock. For the other half of participants, the sizes of the CS+ and oCS- rings were reversed. The generalization stimuli consisted of three rings of intermediate size (i.e., GS_1, GS_2, GS_3) that form a continuum of similarity between the largest and smallest rings. All subjects were also conditioned with the V" shaped safety cue (vCS-) in order to assess generalization to all circular stimuli. Participants may show a fear response to all ring sizes because they are similar in shape to the danger cue. Including vCS-, which is dissimilar in shape to the CS+ and generalization stimuli, allows us to compare brain activations between CS+ and a safety cue that is not influenced by possible generalization effects to all circular stimuli.

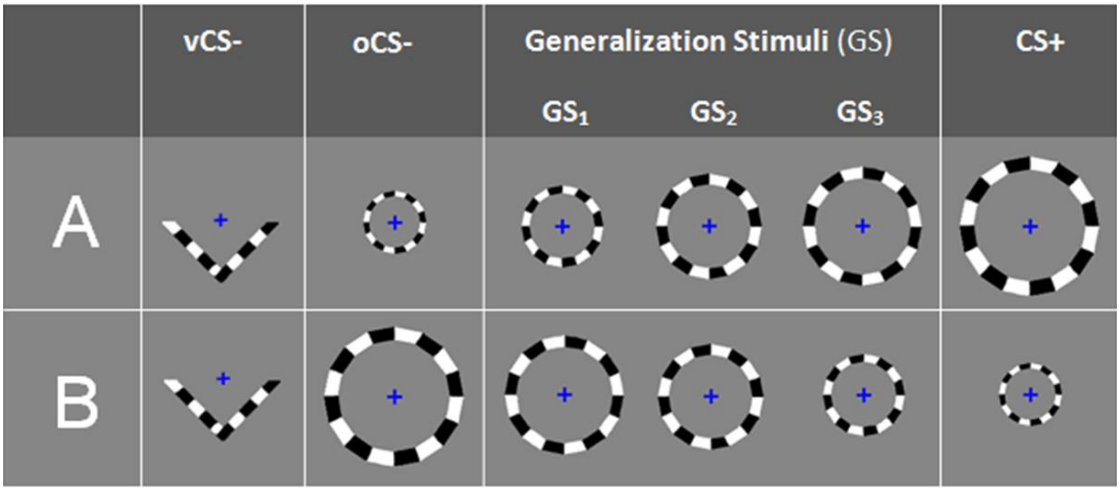

Figure 3.1: Conditioning and generalization stimuli. Counterbalancing groups are designated by A and B (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; GS_1, GS_2 and GS_3 = 3 classes of generalization stimuli; CS+ = conditioned danger cue). Half of participants were assigned to counterbalancing group A and the other half to B. For both counterbalancing groups A and B, GS_3 was the closest in size to CS+, with GS_2 and GS_1 becoming increasingly dissimilar to CS+.

Design

This study consisted of three phases: preacquisition, acquisition, and generalization. All CSs and GSs were presented for 4 s and were projected onto a screen which was reflected onto a mirror attached to the head coil. During inter-trial-intervals (ITIs), participants focused their gaze on crosshairs in the center of the screen. ITIs lasted for either 2.4 or 4.8 s. During the preacquisition phase, CS+, oCS-, vCS-, and the three classes of intermediate ring sizes (GS_1, GS_2, GS_3) were presented 20 times each with no shock reinforcement (120 trials total). During the acquisition phase, CS+, oCS-, and vCS- were presented 20 times each (60 trials total) with 80% of the CS+ stimuli coterminating with an electric shock (100ms, 3-5μA), which was rated by participants as being 'highly uncomfortable but not painful'. Shocks were administered to the participant's right ankle

using a constant current AC stimulator (Precision Instruments). During the generalization phase, each trial type (CS+, oCS-, vCS-, GS_1, GS_2, GS_3) was presented 20 times (120 trials total) with 50% of CS+ coterminating with electric shock.

Behavioral Ratings

Colored crosshairs (blue, yellow, red, green, purple) were presented serially for a duration of 800 ms each in a quasi-random order during stimuli presentation (CSs/GSs). Participants were instructed to continuously monitor the crosshairs and to rate their perceived level of risk for shock (1 = 'no risk', 2 = 'moderate risk', and 3 = 'high risk') as quickly as possible when a red crosshair appeared using a 3 button, fiber optic, response pad (Lumina LP-404 by Cedrus). Risk ratings and reaction times were recorded with Presentation software (Neurobehavioral Systems). The red crosshair appeared on half of the trials. On reinforced CS+ trials, red-crosshairs never coterminated with shock to avoid shocks interfering with behavioral responses. Ratings of anxiety were also collected retrospectively following the pre-acquisition, acquisition, and generalization phases. Risk ratings and retrospective anxiety ratings were transformed into a measure of deviation from linearity (Mean(CS+ and CS-) - Mean (Classes 1, 2, 3, and 4)) in order to obtain a single continuous measure that characterizes the generalization slope which can be correlated with other continuous measures.

Physiological Measurement and Analysis

Skin conductance responses (SCR) were collected using standard methods (Lykken & Venables, 1971). Skin conductance recording was completed using PsychLab software (Precision Instruments, Inc.). Two electrodes were attached to the bottom of the

participant's left foot in order to keep the wires at a safe distance from the radio frequency field of the magnet. SCRs to the stimuli were required to have a latency onset of 1-5 s following stimulus onset. SCRs were calculated by subtracting the onset skin conductance level from the peak skin conductance level of the response wave. SCR data were square root transformed and range corrected to normalize data and to reduce the influence of between subjects variability unrelated to psychological processes (Lykken & Venables, 1971).

fMRI Data Acquisition

A 3T Siemens system equipped with a twelve-channel receive-only head coil was used to acquire functional T2*-weighted echo-planar images (EPIs) depicting the BOLD contrast (TR: 2300 ms, TE: 23 ms, flip: 90°). Whole-brain acquisitions consisted of 36 sagittally-oriented slices of 1.5 mm thickness and 1.5x1.5mm^2 in-plane resolution (matrix: 128×128, FOV: 22 cm). A total of 1144 functional volumes were collected across 5 EPI runs with 235 volumes acquired for runs 1 and 2 (Pre-Acquisition), 170 for run 3 (Acquisition), and 249 and 255 for runs 4 and 5 (Generalization). Three high-resolution T1-weighted magnetization-prepared rapid acquisition gradient-echo sequences (MP-RAGE) were obtained to serve as anatomical reference and to be used in retinotopic mapping (retinotopic data not reported in this paper). Head movement was limited with foam pads during data acquisition.

Procedure

Following informed consent and an MRI safety screening, participants completed a battery of questionnaires including the PTSD Checklist (PCL), the Anxiety Sensitivity

Index (ASI), the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983), the Beck Depression Inventory (BDI; Beck & Steer, 1987), and Fear of Pain Scale (FPS). Next they were interviewed using the Clinician Administered PTSD Scale for the DSM-IV (CAPS; Blake et al., 1990, Blake et al., 1995) and the Structured Clinical Interview for DSM-IV (SCID-I; First et al., 2001). Following the interview, shock electrodes were attached and participants were administered between 1-3 sample shocks. Shock levels were adjusted based on the participant's tolerance. Next, participants practiced the task without shocks before entering the scanner. Participants were told to pay attention to the presented stimuli and that they may be able to predict the shock. No information about the CS/US contingency was provided. Participants were then placed in the scanner. The order of the scans was as follows: first structural scan (MP-RAGE), preacquisition, acquisition, generalization, second MP-RAGE, and third MP-RAGE. Following the completion of each phase (preacquisiton, acquisition, and generalization), participants retrospectively rated their anxiety to CS+, oCS-, and vCS- on a scale from 0 to 10.

fMRI Data Analysis

Image analysis was completed using Analysis of Functional Neural Images (AFNI) software (Cox, 1996). Echo-planar time series data underwent slice-timing correction to adjust the amplitude of the signal acquired from each slice to account for the order of slice acquisition within each volume, motion correction (registration to the seventh volume of the first functional imaging scan), spatial smoothing to minimize the effects of anatomical variability (FWHM= 2.5 mm), and normalization to percent signal

change using as a baseline each subject's voxel-wise time-series mean. Data from separate runs for the same task were concatenated. Subjects with more than 3.0 mm of head motion in any dimension from one EPI brain volume to the next were removed. One participant was excluded from analyses for an abnormal scan.

For the pre-acquisition and generalization phases separately, individual analyses involved computing functional maps by regressing each voxel's fMRI response time-course onto an ideal response function (Gamma-variate function convolved with the time-series of each of 6 stimulus types: vCS-, oCS-, GS_1, GS_2, GS_3, unreinforced CS+). Baseline drift, motion parameters, response time course (button presses), time course of task instructions, and time course of CS+ paired with shock (during generalization only) were modeled as covariates of no interest. The acquisition phase was used to condition participants to CS+, oCS-, and vCS- and was not intended for analyses due to the majority of CS+ trials being contaminated with US administrations. Furthermore, the acquisition data were not critical for testing the generalization hypotheses of interest.

For the generalization-phase data, group-level analyses involved two stages: 1) identification of functional regions of interest (fROI) and 2) averaging beta weights across voxels within these functional ROIs to plot across conditioned and generalization stimuli between groups to analyze group differences in generalization. During stage 1, whole brain analyses were conducted for the CS+ (unreinforced) vs. vCS- contrast using a voxelwise probability of $p \leq .00003$ and a cluster probability of $p \leq .05$. A stringent voxelwise probability was necessary to achieve adequate demarcation between clusters. The AFNI program, 3dClustSim, was used to determine the minimum number of voxels

that define a cluster using the voxelwise and clusterwise *p*-values mentioned above. The CS+ (unreinforced) vs. vCS- contrast was chosen over the CS+ (unreinforced) vs. oCS- contrast because the latter contrast is influenced by the possible generalization of fear to all circular stimuli. However, the pattern of generalization results does not change significantly when analyzing the CS+ (unreinforced) vs. oCS- contrast.

During stage 2 of the group-level analyses, average beta weights for functional ROIs were plotted for the conditioned and generalization stimuli separately for each group. Analysis of generalization effects utilized 4x6 repeated measures ANOVAs (four groups: PTSD, sub-threshold PTSD, trauma control, non-trauma control by six stimuli: vCS, oCS-, GS_1, GS_2, GS_3, unreinforced CS+). ANOVAs were computed using Wilks's lambda and were followed, when necessary, by quadratic and linear trend analyses and/or paired-samples t tests. Criterion alpha for ANOVAs and follow up statistics was set at $p = .05$. Geisser-Greenhouse corrections were used when there were violations of the sphericity assumption.

Functional Connectivity Analysis

Psychophysiological interaction (PPI) was used to estimate the connectivity between brain regions in relation to the generalization task (Friston et al., 1997). Functionally defined seed regions included the hippocampus, right and left insula, vmPFC, and dmPFC based on a priori predictions regarding the fear-conditioning neural network (see Lissek et al., 2013). We would predict increased neural connectivity between the hippocampus and brain areas associated with fear excitation (e.g., insula) when comparing stimuli with more versus less resemblance to CS+ and increased neural

connectivity between the hippocampus and brain areas associated with fear inhibition (e.g., vmPFC) when comparing stimuli with less versus more resemblance to CS+. The criterion alpha was set at $p \leq 0.001$ based on previous research employing PPI analyses (Passamonti et al., 2009).

Results

Subjective Ratings and Reaction Times

Pre-acquisition. No differences were found between CS+ and oCS- for retrospective ratings of anxiety (p = .36) or online behavioral ratings of perceived risk for shock (p = .44) prior to conditioning. In addition, there were no differences between CS+ and vCS- for online behavioral ratings of perceived risk for shock (p = .33) during pre-acquisition. However, retrospective ratings of anxiety were higher for vCS- than CS+ prior to conditioning (vCS- M=2.75, SD=2.87; CS+ M=1.89, SD=2.32; $t(78)$= 3.05, p = .003). In addition, a main effect for group was apparent for retrospective anxiety ratings ($F(3,75)$= 3.41, p = .022). Further analyses revealed higher retrospective ratings of anxiety to vCS- than CS+ in the non-trauma control group (p = .029) and in the PTSD group, although this did not reach significance (p = .061). The trauma control and sub-threshold PTSD groups did not differ in retrospective ratings of anxiety between vCS- and CS+ (p-values ≥ .19). The conditioned and generalization stimuli did not differ in online risk ratings regardless of whether the vCS- is included or excluded (p-values ≥ .22) and there were no group differences in online risk ratings for any of the stimuli ($p \geq$ .33). No differences in reaction times were found for any of the stimuli during pre-acquisition (p-values ≥ .13).

Acquisition. Following conditioning, online ratings of risk were greater to CS+ (*M*=1.40, *SD*=.55) than to oCS- (*M*=.40, *SD*=.53) or vCS- (*M*=.36, *SD*=.50), *p*-values < .0001, suggesting that the participants successfully acquired conditioned fear to the danger cue. In addition, retrospective ratings of anxiety were greater for CS+ (*M*=6.43, *SD*=2.69) compared to both oCS- (*M*=1.75, *SD*=2.26) and vCS- (*M*=1.76, *SD*=2.40), *p*-values < .0001. Online risk ratings and retrospective anxiety ratings did not differ between oCS- and vCS- (*p*-values ≥ .39). Figures 3.2 and 3.3 show retrospective anxiety ratings and risk ratings as a function of stimulus type and group. No main effects for group or group by stimulus interactions were found for online risk ratings or retrospective anxiety (*p*-values ≥ .21); however, a group by stimulus type interaction was found for reaction times ($F(6,148)=2.86$, $p = .012$). The non-trauma control group showed faster reaction times to oCS- (*M*=5309.21, *SD*=743.86) than vCS- (*M*=6126.97, *SD*=1306.27; $p = .007$) or CS+ (*M*=6204.18, *SD*=1245.82; $p = .005$). The sub-threshold PTSD group showed a trend toward faster reaction times to oCS- (*M*=5966.47, *SD*=1198.36) compared to CS+ (*M*=6626.83, *SD*=2045.98; $p = .058$). No other group differences in reaction times were significant (*p*-values ≥ .28).

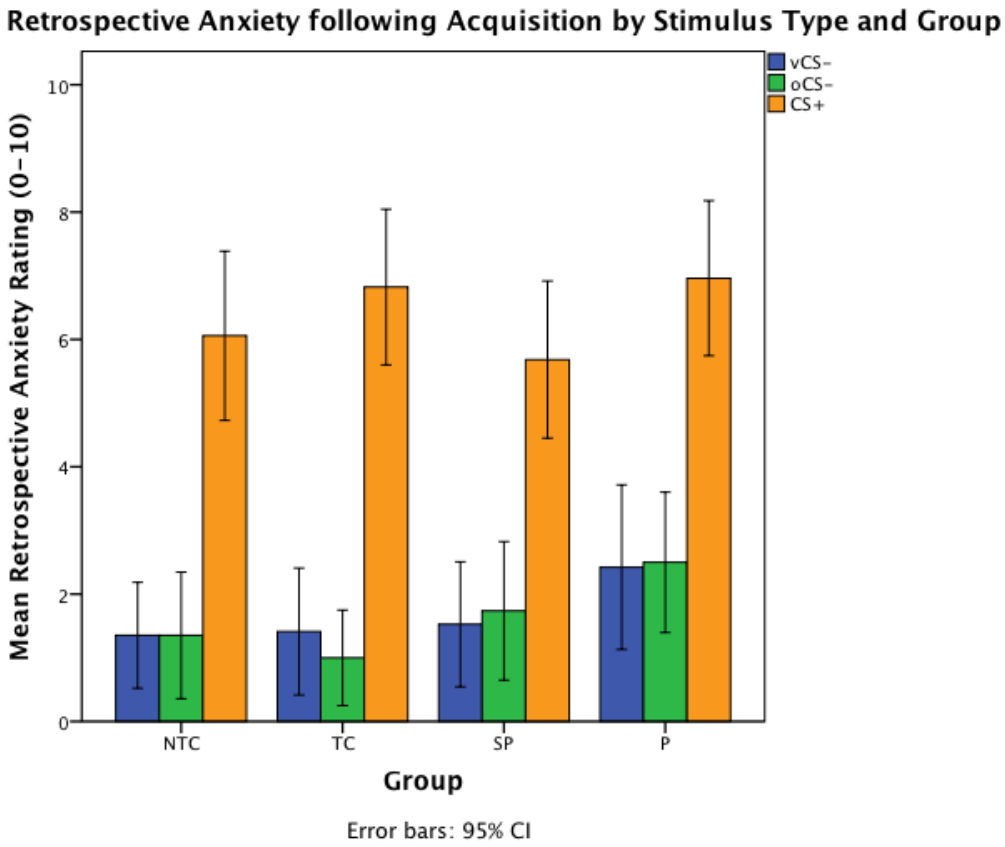

Figure 3.2: Retrospective anxiety ratings (scale of 0-10) following the acquisition phase as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; CS+ = conditioned danger cue) and group (NTC = non-trauma controls, TC = trauma controls, SP = sub-threshold PTSD group, P = PTSD patients).

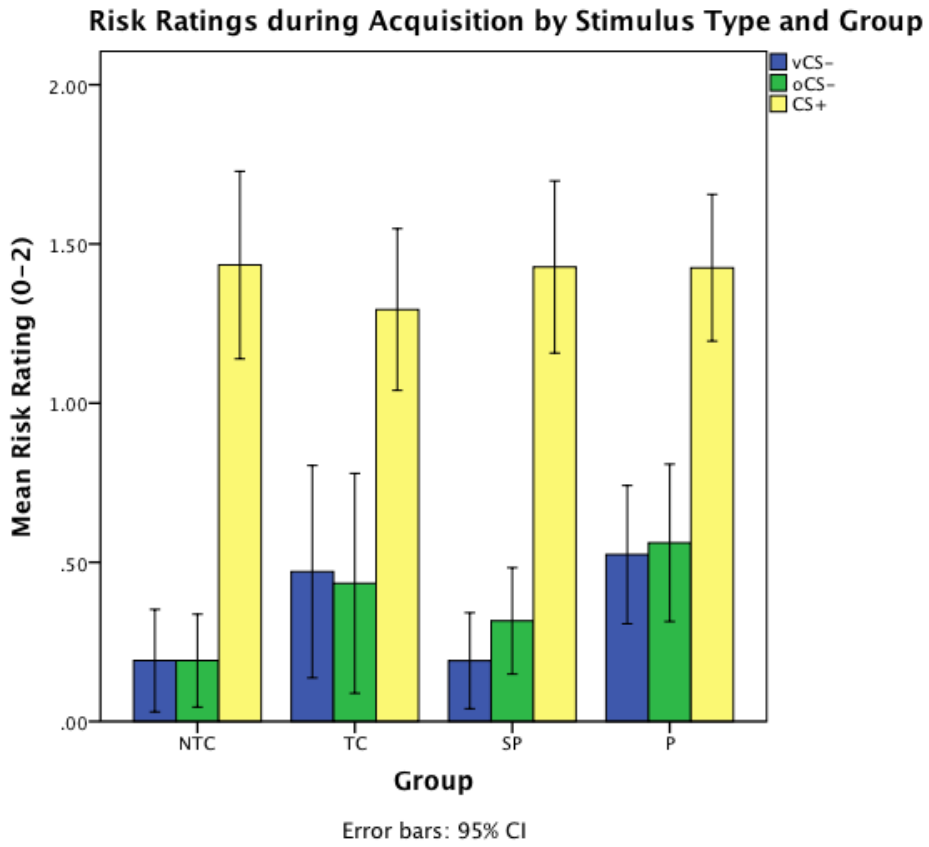

Figure 3.3: Risk ratings (0 = no risk, 1 = some risk, 2 = a lot of risk) during the acquisition phase as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; CS+ = conditioned danger cue) and group (NTC = non-trauma controls, TC = trauma controls, SP = sub-threshold PTSD group, P = PTSD patients).

Generalization. Online risk ratings and retrospective anxiety continued to be greater to CS+ versus both oCS- and vCS- (all *p*-values < .0001), suggesting that conditioned fear persisted during the generalization sequence. No group differences in online risk ratings and retrospective anxiety to CS+ versus both oCS- and vCS- were apparent (*p*-values ≥ .39). A 4x6 (group by stimulus type) repeated measures ANOVA revealed a significant main effect for stimulus type for the online risk ratings ($F(5,71)=40.45$, $p <$

.0001). Levels of reported risk increased from vCS- to oCS- to $GS_{1,}$ to $GS_{2,}$ to GS_3 to CS+ (see Figures 3.4a-d), suggesting generalization of conditioned fear as the stimulus becomes more similar to CS+. No main effect for group or group by stimulus interaction was found for online risk ratings (p-values $\geq .28$). All groups showed generalization gradients consisting of both linear (p-values $< .0001$) and quadratic components (non-trauma controls: $p < .001$; trauma controls: $p < .001$; sub-threshold PTSD group: $p = .001$; PTSD group: $p = .008$).

Planned contrasts corrected for multiple comparisons using Bonferroni's method (criterion $p = .003$) showed significantly elevated levels of risk to GS_3 and CS+ relative to vCS- in the non-trauma and trauma control groups (p-values $< .001$; denoted with a black marker in Figures 3.4a-d). The sub-threshold PTSD and PTSD groups showed significantly elevated levels of risk to GS_2, GS_3, and CS+ relative to vCS- (p-values $\leq .001$), suggesting generalization of perceived risk to a greater range of stimuli in these groups. For the reaction times, a significant main effect for stimulus type was found ($p < .0001$). Reaction times were characterized by an inverted U shape with slower reaction times to the generalization stimuli (see Figure 3.5), consistent with previous research showing slower responding for stimuli with less certain threat information than the conditioned danger and safety cues (Lissek et al., 2010). No group by stimulus type interaction or main effect of group was found for reaction times (p-values $\geq .40$).

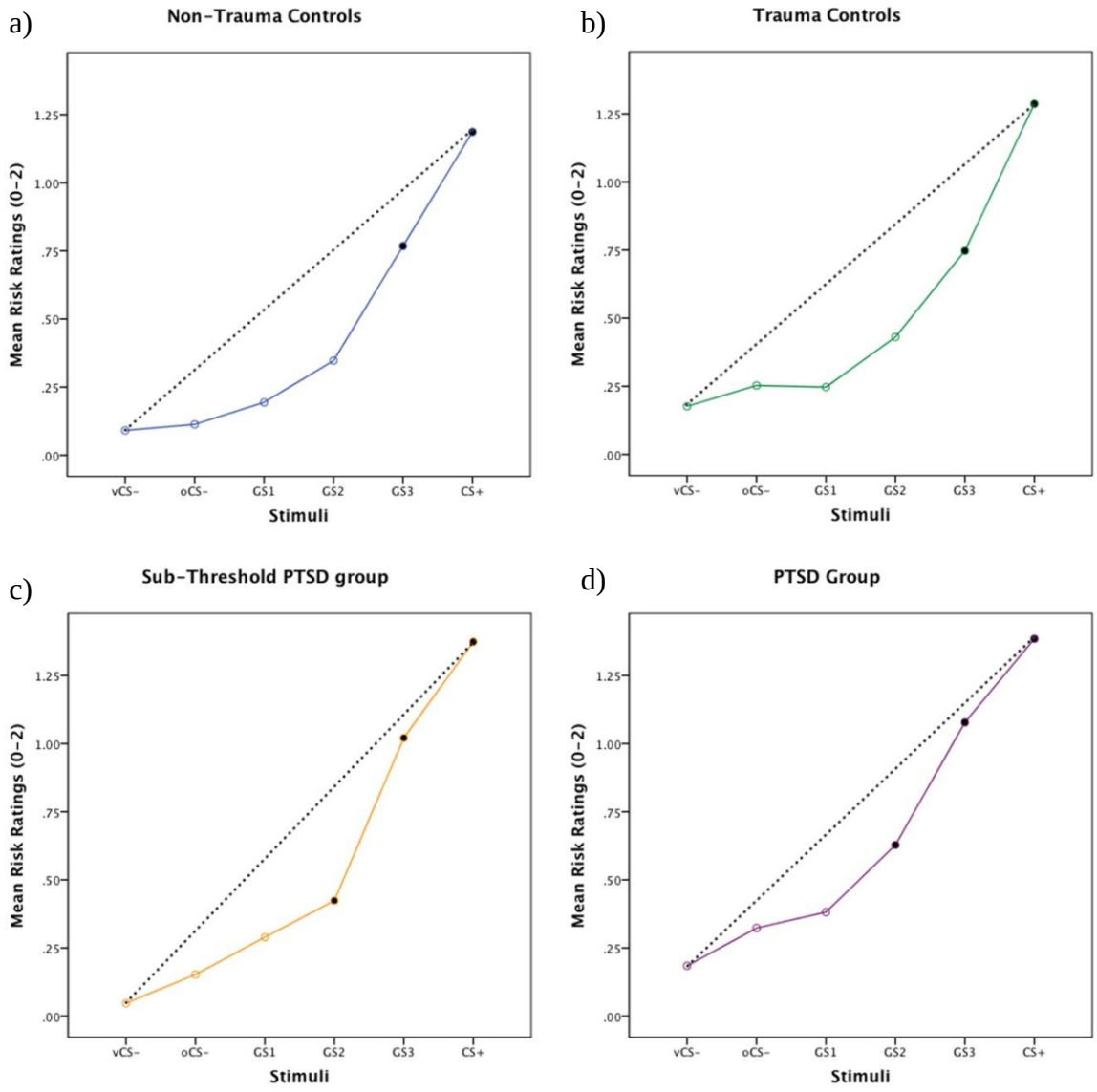

Figure 3.4a-d: Risk ratings (0 = no risk, 1 = some risk, 2 = a lot of risk) for each group during the generalization phase as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; GS1, GS2, GS3 = generalization stimuli; CS+ = conditioned danger cue). Dotted lines reflecting linear decreases in risk from CS+ to vCS- are included to illustrate each gradient's deviation from linearity. Black dots indicate a significant difference in risk ratings when compared to vCS-.

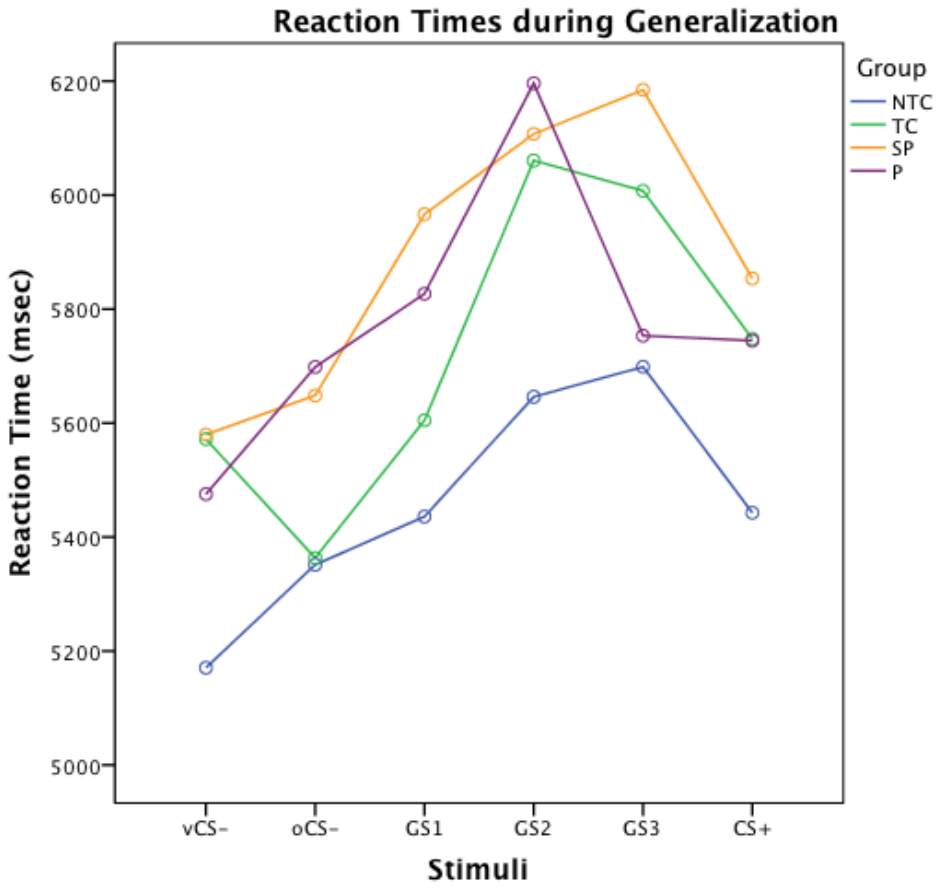

Figure 3.5: Reaction times (msec) during the generalization phase as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; GS1, GS2, GS3 = generalization stimuli; CS+ = conditioned danger cue) and group (NTC = non-trauma controls, TC = trauma controls, SP = sub-threshold PTSD group, P = PTSD patients).

Skin Conductance

Pre-acquisition. During pre-acquisition, no main effect for stimulus type, main effect for group, or stimulus type by group interaction was found, suggesting no differences in skin conductance prior to conditioning.

Acquisition. A repeated measures ANOVA revealed a significant main effect for stimulus type ($F(2,73)=7.90$, $p = .001$). Greater skin conductance responses were found to CS+ ($M=.64$, $SD=.38$) than to oCS- ($M=.44$, $SD=.32$) or vCS- ($M=.46$, $SD=.30$) p-values $\leq .001$ (see Figure 3.6), further suggesting the successful acquisition of conditioned fear following conditioning. No main effect for group or group by stimulus type interaction was found (p-values $\geq .16$; see Figure 3.7).

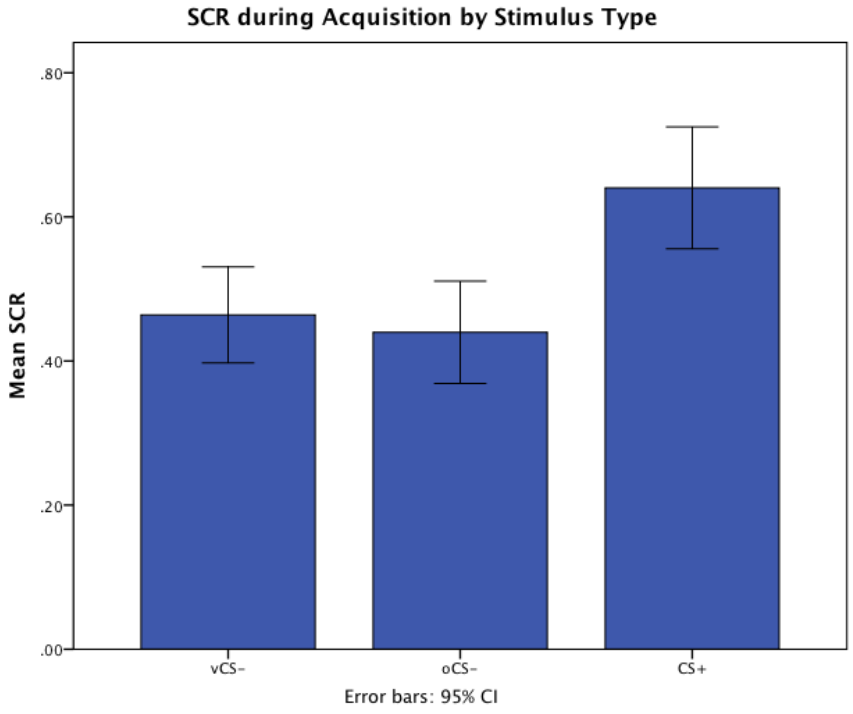

Figure 3.6: Skin conductance responses (SCR) during the acquisition phase for each stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; CS+ = conditioned danger cue).

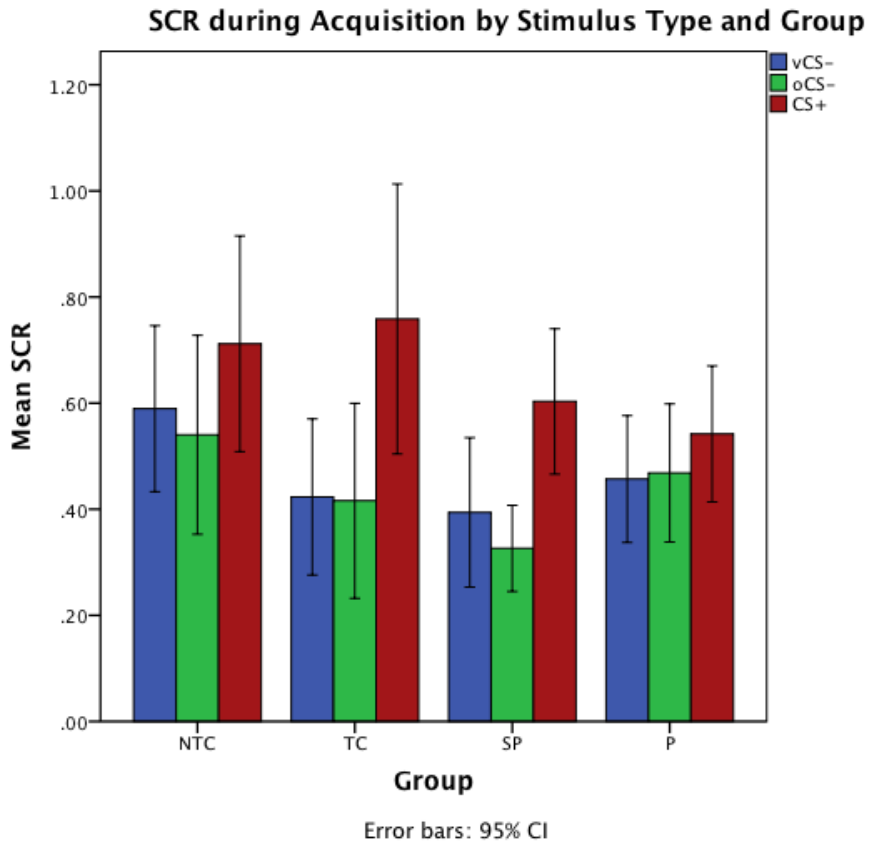

Figure 3.7: Skin conductance responses (SCR) during the acquisition phase as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; CS+ = conditioned danger cue) and group (NTC = non-trauma controls, TC = trauma controls, SP = sub-threshold PTSD group, P = PTSD patients).

Generalization. A repeated measures ANOVA revealed a significant main effect for stimulus type ($F(5,68)=13.56$, $p < .001$). Figure 3.8 shows the skin conductance gradients where skin conductance responses were greatest to CS+ and smaller for the other stimuli, suggesting that conditioned fear persisted during generalization. No main effect for group or group by stimulus interaction was found (p-values $\geq .46$). Gradients were characterized by linear slopes in the trauma controls, sub-threshold PTSD group, and PTSD group (p-values $\leq .046$) and cubic components in the non-trauma controls, trauma controls, and

PTSD group (p-values ≤ .037). There was a trend toward a quadratic slope in the non-trauma controls (p = .085), but no other groups showed quadratic components (p-values ≥ .20).

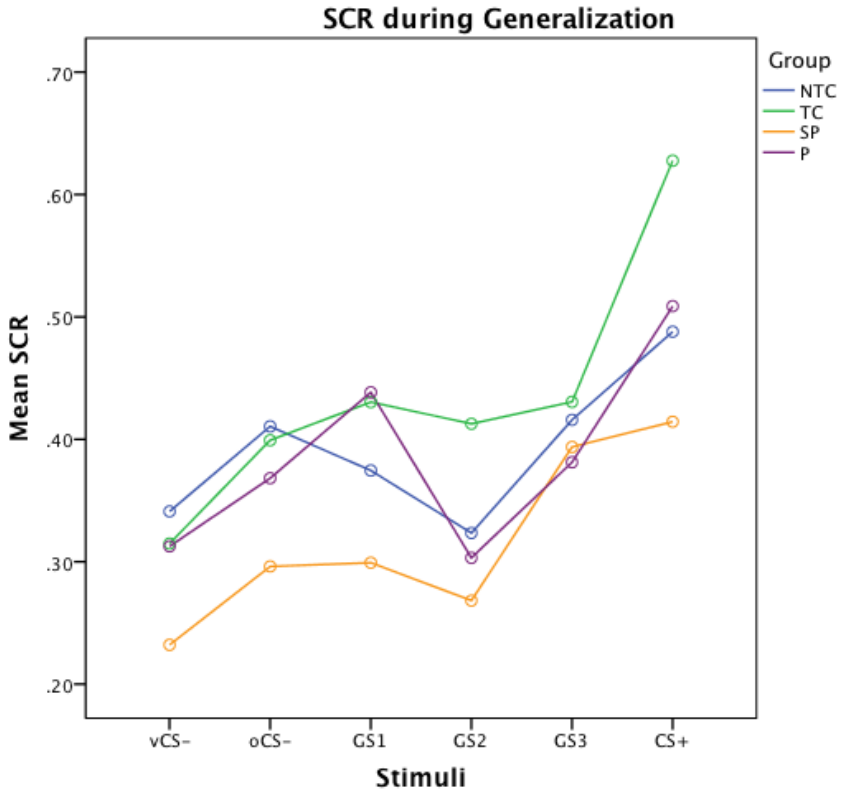

Figure 3.8: Skin conductance responses (SCR) during the generalization phase as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; GS1, GS2, GS3 = generalization stimuli; CS+ = conditioned danger cue) and group (NTC = non-trauma controls, TC = trauma controls, SP = sub-threshold PTSD group, P = PTSD patients).

fMRI Activations

Functional regions of interest (fROI's). Table 3.2 lists the brain regions that were differentially activated to CS+ versus vCS- and that survived a voxelwise probability of

$3*10^{-5}$ and a cluster probability of .05. Using these brain areas as functional ROI's, percent signal change was measured for each stimulus type (vCS-, oCS-, G_1, G_2, G_3, CS+) and generalization gradients were compared before and after acquisition training.

Pre-acquisition. Prior to conditioning, these fROI's did not show linear or quadratic increases in BOLD signal as the presented stimulus increased in similarity to the CS+.

Generalization test. Following acquisition, 4x6 (group by stimuli) repeated measures ANOVAs revealed significant group by stimulus type interactions in the right insula ($F(15,193.64)=2.45$, $p = .003$), left insula ($F(15,193.64)=1.77$, $p = .042$), dmPFC ($F(15,193.64)=2.62$, $p = .001$), right precentral gyrus (BA9: $F(15,193.64)=2.41$, $p = .003$), and left inferior parietal lobule ($F(15,193.64)=1.94$, $p = .022$). Furthermore, tests of within subjects effects revealed significant group by stimulus type interactions in the left caudate nucleus ($F(11.81,291.30)=1.96$, $p = .029$), right caudate nucleus ($F(10.78,265.91)=1.94$, $p = .036$), left thalamus ($F(12.93,318.86)=1.79$, $p = .044$), right thalamus ($F(12.33,304.13)=1.80$, $p = .046$), and right superior frontal gyrus (BA 10; $F(15,370)=1.87$, $p = .025$). Specifically, these areas were associated with generalization gradients characterized by *positive* slopes in BOLD activations (responses strongest to CS+ with degraded reactivity to GS_3, GS_2, GS_1, oCS-, and vCS-).

Table 3.2
Brain areas responding differentially to CS+ versus vCS- that served as functional regions of interest (fROIs).

Brain Region	Direction	Volume	Peak Coordinates*			ß
			X	Y	Z	
dmPFC (BA6)	CS+ > vCS-	10438.88	-1.5	-16.5	35.5	0.29
R insula	CS+ > vCS-	7074	-52.5	-16.5	1.0	0.34
L insula	CS+ > vCS-	5936.63	48.0	-16.5	-.05	0.30
R PreCG	CS+ > vCS-	3918.38	-45.0	-6.0	34.0	0.25
R caudate	CS+ > vCS-	1434.38	-7.5	-3.0	8.5	0.34
L caudate	CS+ > vCS-	1589.63	6.0	-4.5	7.0	0.34
R thalamus	CS+ > vCS-	1586.25	-1.5	21.0	1.0	0.29
L thalamus	CS+ > vCS-	833.63	6.0	16.5	11.5	0.26
R LG (BA18)	CS+ > vCS-	803.25	-13.5	82.5	-9.5	0.45
L LG (BA18)	CS+ > vCS-	941.63	13.5	84.0	-12.5	0.41
R SFG (BA10)	CS+ > vCS-	651.38	-34.5	-51.0	23.5	0.19
R IPL	CS+ > vCS-	567	-61.5	42.0	22.0	0.20
L IPL	CS+ > vCS-	256.5	61.5	24.0	23.5	0.24
vmPFC	vCS- > CS+	4053.38	6.0	-31.5	-8.0	0.31
PCu	vCS- > CS+	1701	1.5	51.0	17.5	0.22
R hippocampus	vCS- > CS+	135	-22.5	16.5	-11.0	0.35
L hippocampus	vCS- > CS+	114.75	21.0	16.5	-9.5	0.35
R MTG	vCS- > CS+	236.25	-48.0	66.0	23.5	0.19
L MTG	vCS- > CS+	1761.75	42.0	72.0	34.0	0.22
R ITG (BA19)	vCS- > CS+	722.25	-54.0	64.5	-0.5	0.27
L SFG (BA 9)	vCS- > CS+	280.13	9.0	-60.0	29.5	0.20
R CG	vCS- > CS+	236.25	-18.0	-6.0	25.0	0.24

Note. ß = mean beta weight; CS+ = conditioned danger cue; vCS- = v-shaped conditioned safety cue; L = left; R = right; BA = Brodmann Area; dmPFC = dorsomedial prefrontal cortex; PreCG = precentral gyrus; LG = lingual gyrus; SFG = superior frontal gyrus; IPL = inferior parietal lobule; vmPFC = ventromedial prefrontal cortex; PCu = precuneus; MTG = middle temporal gyrus; ITG = inferior temporal gyrus; CG = cingulate gyrus.
*Peak coordinates are in RAI format.

Tests of linear and quadratic trends were used to examine for group differences in the steepness of the generalization gradient slopes. Analyses revealed that non-trauma controls, trauma controls, and the sub-threshold PTSD group were associated with both linear and quadratic trends in dmPFC, bilateral insula, and right caudate nucleus (*p*-values ≤ .023), indicating that as the stimulus differs from the danger cue, there is a steep drop in BOLD activation (see Figures 3.9-3.12). Conversely, the PTSD group's generalization gradients were associated with linear trends (*p*-values ≤ .0001) but no significant quadratic trends (*p*-values ≥ .082) in these areas, suggesting a less precipitous decline in BOLD activation as the stimulus decreases in similarity to CS+ (greater generalization). The left caudate nucleus was associated with linear and quadratic trends in the non-trauma controls and trauma controls (*p*-values ≤ .008) compared to the sub-threshold PTSD and PTSD groups, which showed linear trends (*p*-values ≤ .002) but no quadratic trends (*p*-values ≥ .19; see Figure 3.13). Additional positive gradients were found in the right precentral gyrus (BA9), left and right thalamus, left inferior parietal lobule, and right superior frontal gyrus (BA 10), which were associated with linear trends for all groups (*p*-values ≤ .037) and quadratic trends only in the trauma controls (*p*-values ≤ .026).

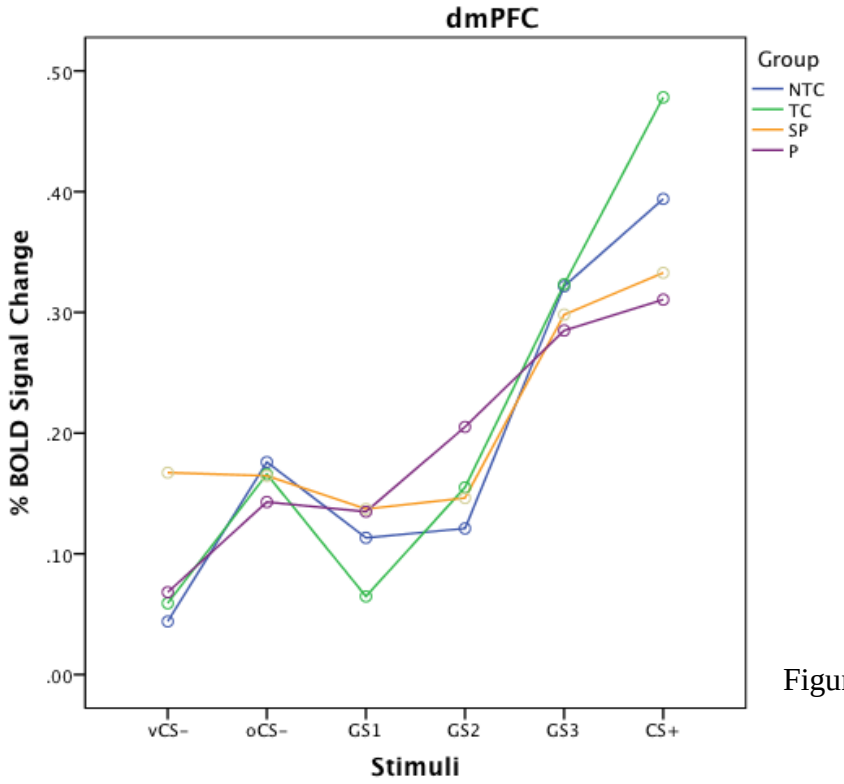

Figure 3.9a

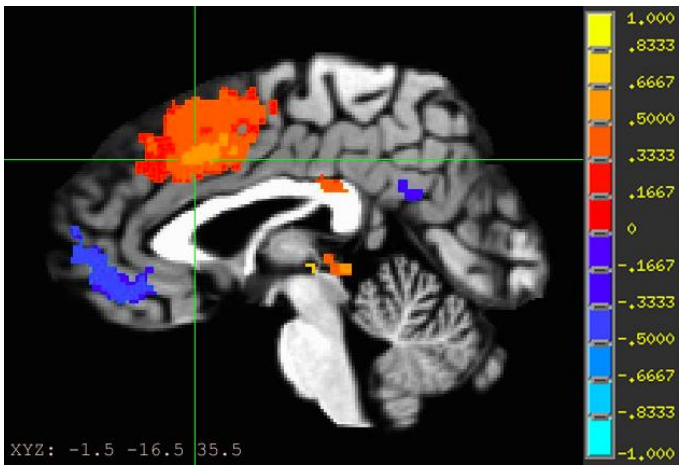

Figure 3.9b

Figure 3.9: a) Percent BOLD signal change in the dorsal medial prefrontal cortex as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; GS1, GS2, GS3 = generalization stimuli, CS+ = conditioned danger cue) and group (NTC = non-trauma controls, TC = trauma controls, SP = sub-threshold PTSD group, P = PTSD patients); b) activation in the dorsal medial prefrontal cortex (peak voxel denoted with crosshairs).

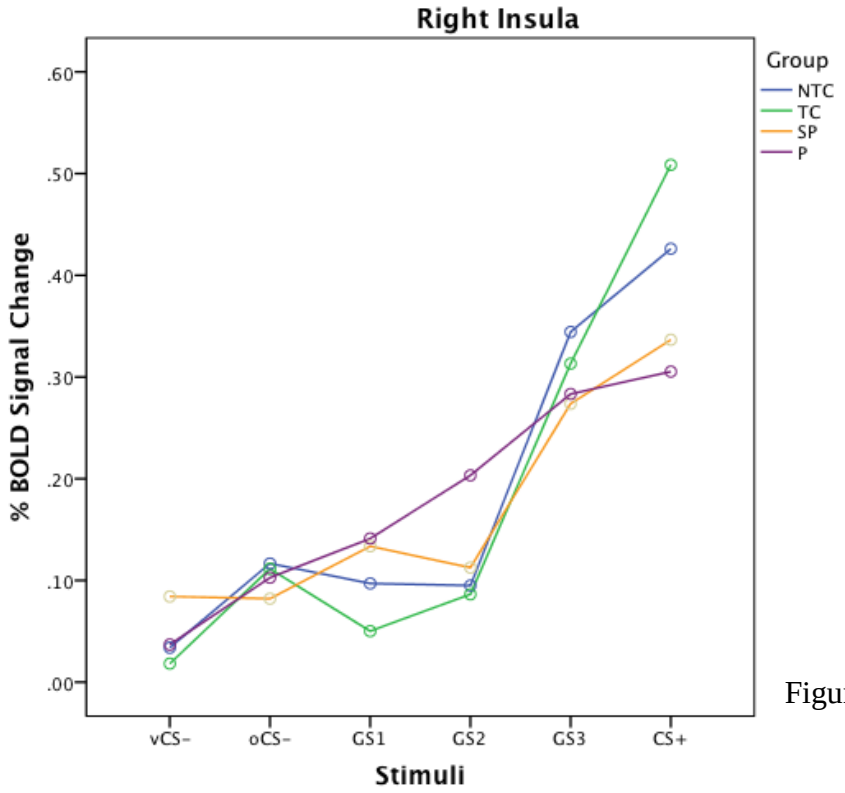

Figure 3.10a

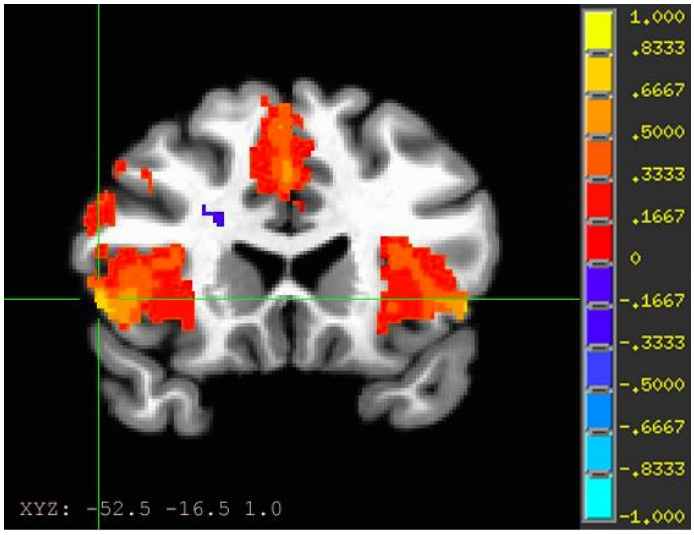

Figure 3.10b

Figure 3.10: a) Percent BOLD signal change in the right insula as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; GS1, GS2, GS3 = generalization stimuli, CS+ = conditioned danger cue) and group (NTC = non-trauma controls, TC = trauma controls, SP = sub-threshold PTSD group, P = PTSD patients); b) activation in the right insula (peak voxel denoted with crosshairs).

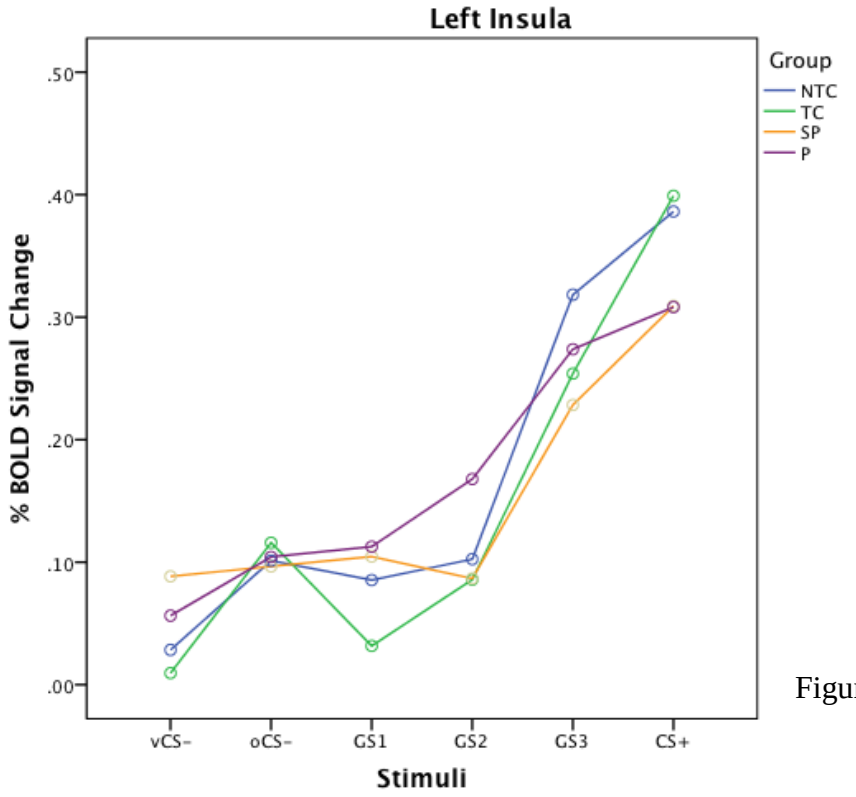

Figure 3.11a

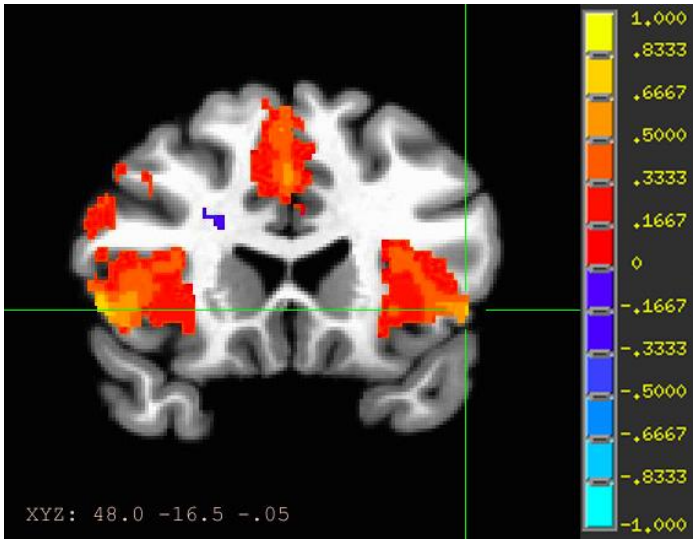

Figure 3.11b

Figure 3.11: a) Percent BOLD signal change in the left insula as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; GS1, GS2, GS3 = generalization stimuli, CS+ = conditioned danger cue) and group (NTC = non-trauma controls, TC = trauma controls, SP = sub-threshold PTSD group, P = PTSD patients); b) activation in the left insula (peak voxel denoted with crosshairs).

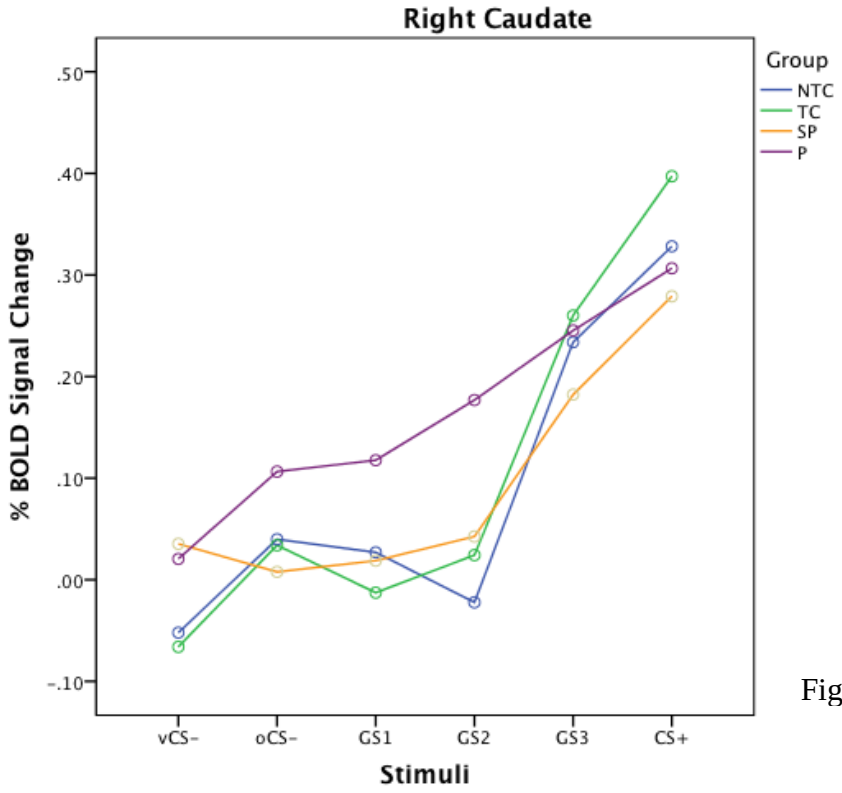

Figure 3.12a

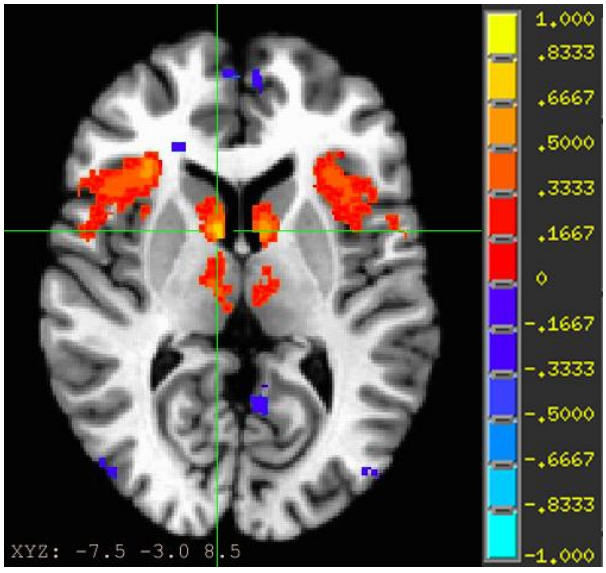

Figure 3.12b

Figure 3.12: a) Percent BOLD signal change in the right caudate as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; GS1, GS2, GS3 = generalization stimuli, CS+ = conditioned danger cue) and group (NTC = non-trauma controls, TC = trauma controls, SP = sub-threshold PTSD group, P = PTSD patients); b) activation in the right caudate (peak voxel denoted with crosshairs).

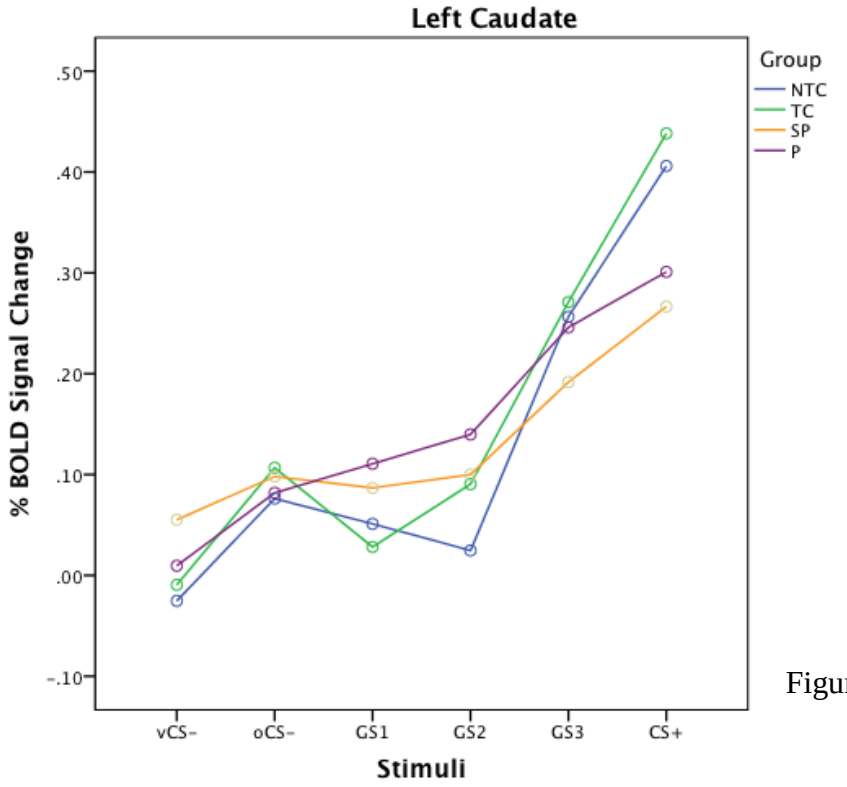

Figure 3.13a

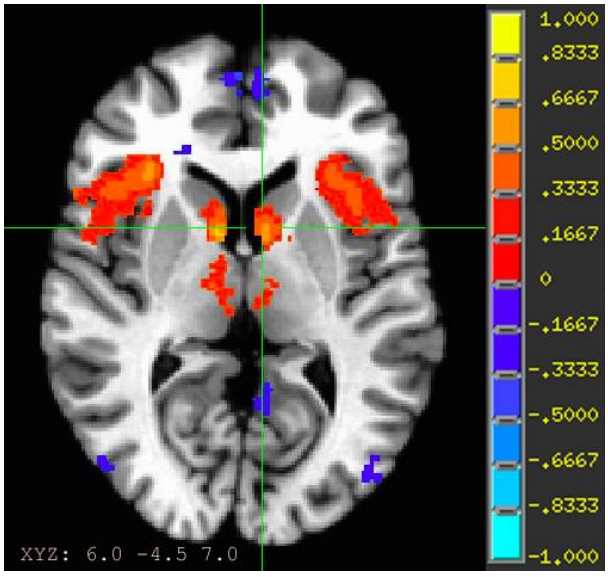

Figure 3.13b

Figure 3.13: a) Percent BOLD signal change in the left caudate as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; GS1, GS2, GS3 = generalization stimuli, CS+ = conditioned danger cue) and group (NTC = non-trauma controls, TC = trauma controls, SP = sub-threshold PTSD group, P = PTSD patients); b) activation in the left caudate (peak voxel denoted with crosshairs).

No group differences were found in the other regions listed in table 3.2 but the following fROIs were also associated with positive generalization gradient slopes: right lingual gyrus (BA18), left lingual gyrus (BA18), and right inferior parietal lobule (linear: p-values $\leq .0001$, quadratic: p-values $\leq .0001$). Areas associated with negative generalization gradients (responses strongest to vCS- with degraded reactivity to oCS-, GS_1, GS_2, GS_3. and CS+) but no group differences included: ventromedial prefrontal cortex (linear: $p < .0001$; quadratic: $p = .028$), left hippocampus (linear: $p < .0001$; quadratic: $p = .005$), right hippocampus (linear: $p < .0001$; quadratic: $p = ns$), and precuneus (linear: $p < .0001$; quadratic: $p = ns$), see Figures 3.14-3.17. No group differences were apparent in any of these areas when comparing the PTSD group to non-trauma controls only (p-values $\geq .28$) or to trauma controls only (p-values $\geq .31$). Additional areas associated with negative generalization gradients included left and right middle temporal gyrus (linear: p-values $< .0001$; quadratic: p-values = ns), right inferior temporal gyrus (linear: $p < .0001$; quadratic: $p = .002$), left superior frontal gyrus (linear: $p \leq .0001$, quadratic: $p = ns$), and right cingulate gyrus (linear: $p < .0001$; quadratic: $p = .001$).

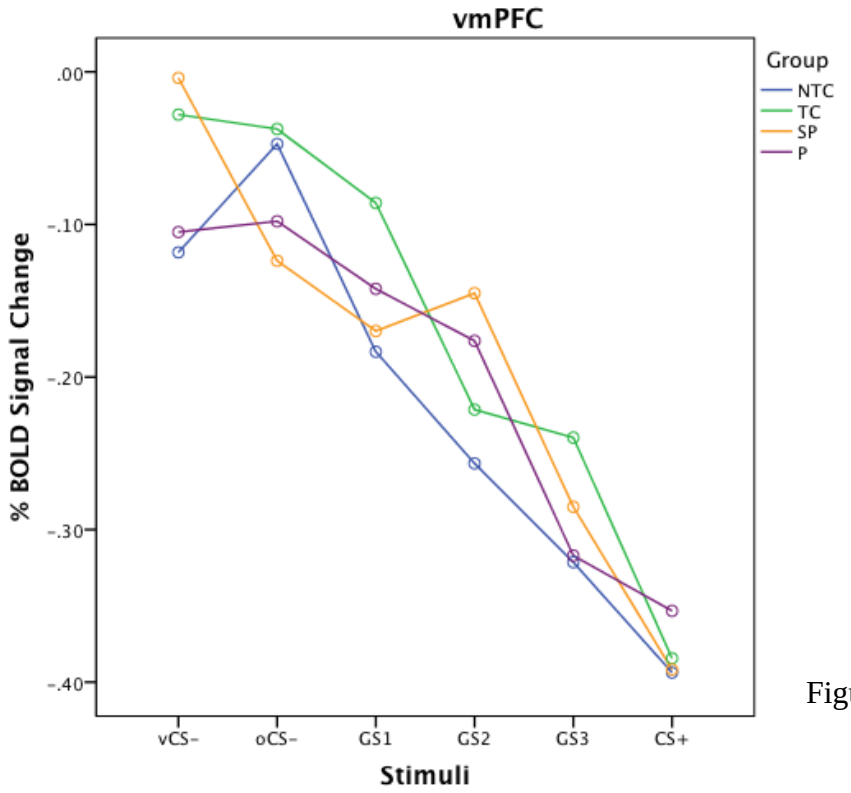

Figure 3.14a

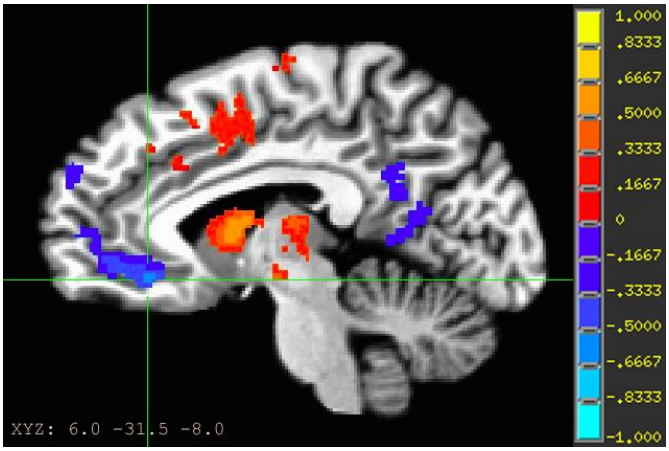

Figure 3.14b

Figure 3.14: a) Percent BOLD signal change in vmPFC as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; GS1, GS2, GS3 = generalization stimuli, CS+ = conditioned danger cue) and group (NTC = non-trauma controls, TC = trauma controls, SP = sub-threshold PTSD group, P = PTSD patients); b) activation in vmPFC (peak voxel denoted with crosshairs).

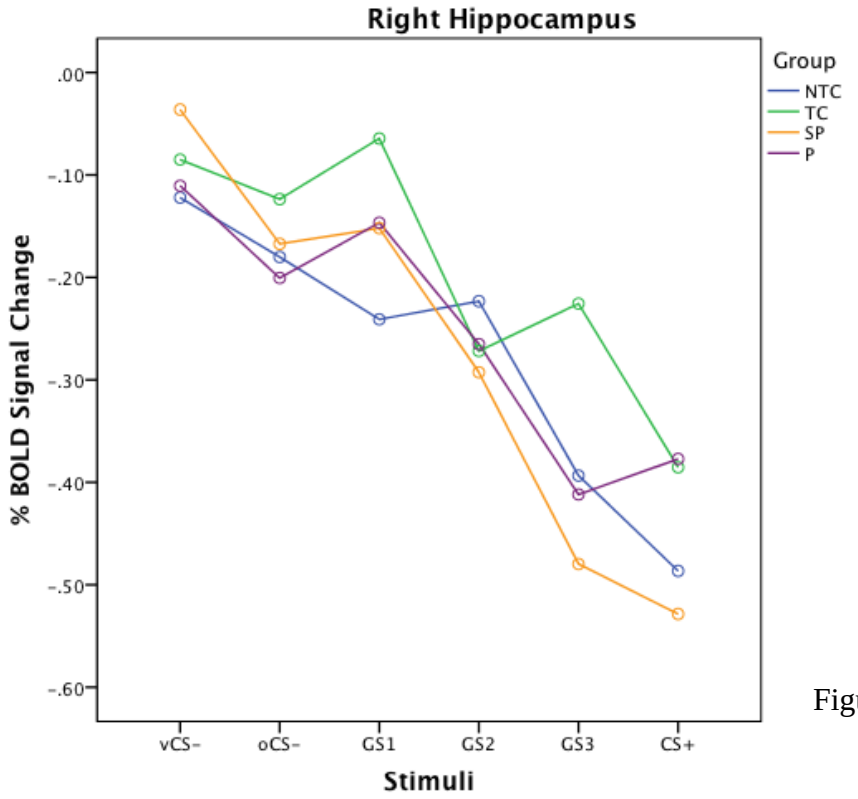

Figure 3.15a

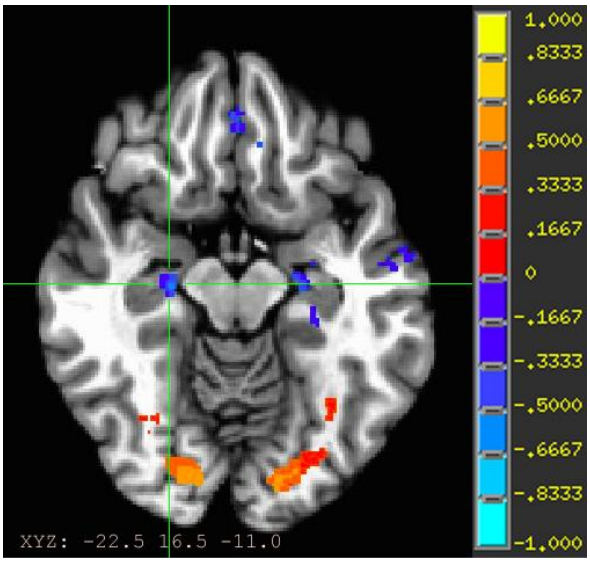

Figure 3.15b

Figure 3.15: a) Percent BOLD signal change in right hippocampus as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; GS1, GS2, GS3 = generalization stimuli, CS+ = conditioned danger cue) and group (NTC = non-trauma controls, TC = trauma controls, SP = sub-threshold PTSD group, P = PTSD patients); b) activation in right hippocampus (peak voxel denoted with crosshairs).

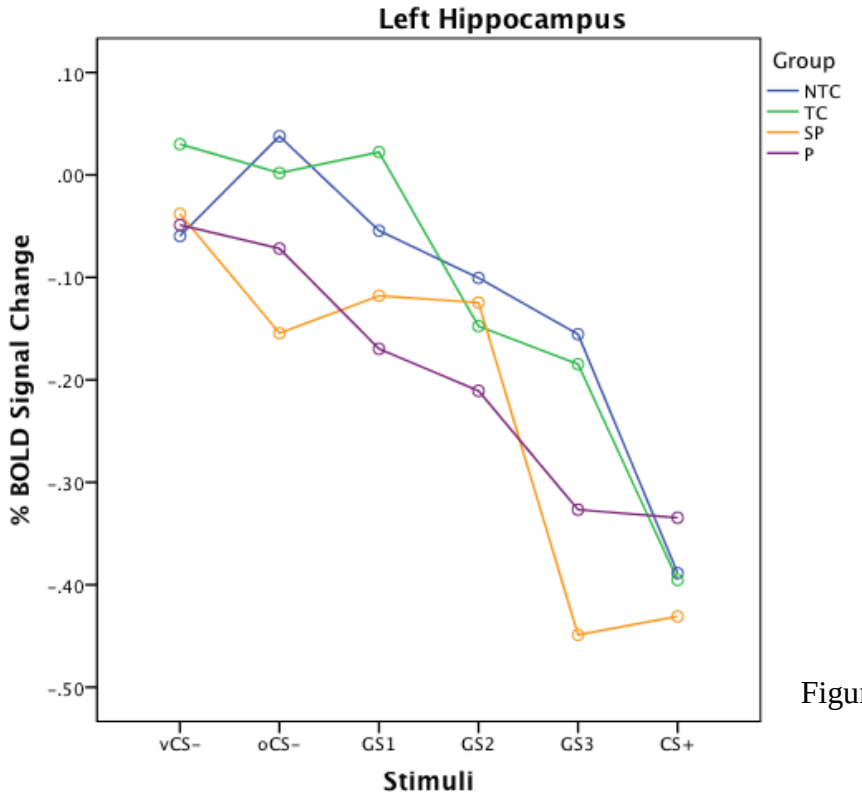

Figure 3.16a

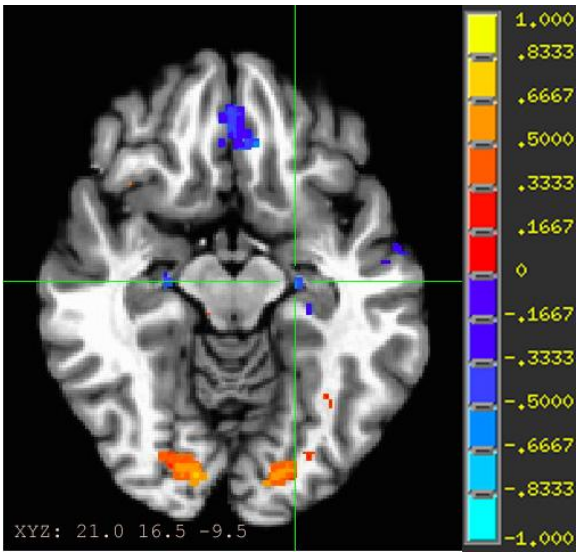

Figure 3.16b

Figure 3.16: a) Percent BOLD signal change in left hippocampus as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; GS1, GS2, GS3 = generalization stimuli, CS+ = conditioned danger cue) and group (NTC = non-trauma controls, TC = trauma controls, SP = sub-threshold PTSD group, P = PTSD patients); b) activation in left hippocampus (peak voxel denoted with crosshairs).

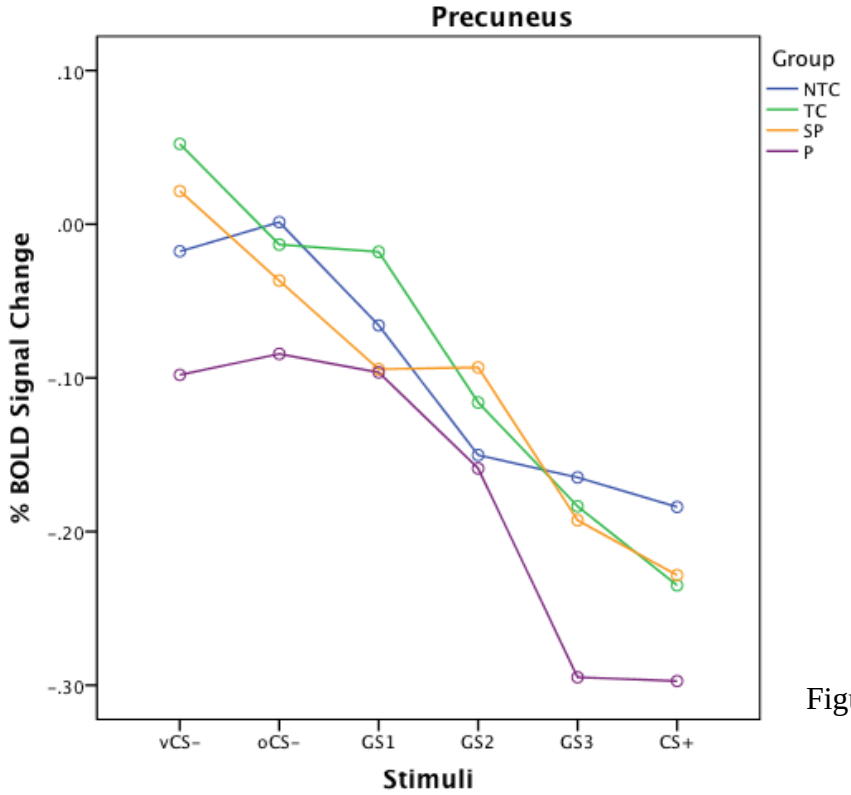

Figure 3.17a

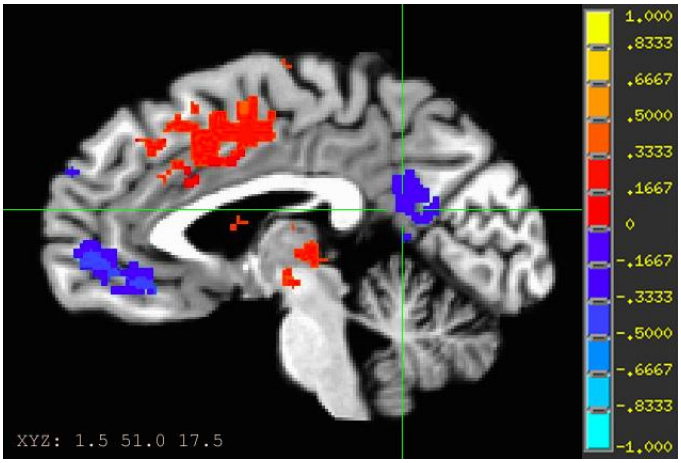

Figure 3.17b

Figure 3.17: a) Percent BOLD signal change in precuneus as a function of stimulus type (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; GS1, GS2, GS3 = generalization stimuli, CS+ = conditioned danger cue) and group (NTC = non-trauma controls, TC = trauma controls, SP = sub-threshold PTSD group, P = PTSD patients); b) activation in precuneus (peak voxel denoted with crosshairs).

Brain-Behavior Correlations

Conditioned fear. Online ratings of perceived shock risk and retrospective anxiety ratings were correlated with the average activations in each of the fROIs. Risk ratings to CS+ versus vCS- were positively correlated with CS+ minus vCS- BOLD activations in the right insula ($r(76)= .32$, $p = .004$), left insula ($r(76)= .26$, $p = .020$), and right caudate ($r(76)= .24$, $p = .032$), and negatively correlated with the vmPFC ($r(76)= -.37$, $p = .001$) and precuneus ($r(76)= -.28$, $p = .014$). Retrospective anxiety ratings to CS+ versus vCS- were positively correlated with activations in the dmPFC ($r(76)= .24$, $p = .036$), right insula ($r(76)= .28$, $p = .012$), left insula ($r(76)= .33$, $p = .003$), right precentral gyrus ($r(76)= .28$, $p = .012$), right thalamus ($r(76)= .26$, $p = .023$), and left thalamus ($r(76)= .28$, $p = .013$), and negatively correlated with the vmPFC ($r(76)= -.34$, $p = .003$), left middle temporal gyrus ($r(76)= -.23$, $p = .046$), and precuneus ($r(76)= -.25$, $p = .029$). No other fROI correlations were significant.

Generalization. Brain-behavior relations for conditioned generalization were tested using a measure of deviation from linearity calculated for risk ratings and BOLD activations as (Mean(CS+ and vCS-) - Mean (GS_1, GS_2, and GS_3)) in order to obtain a single continuous measure that characterizes the shape of the generalization slope. Using this measure, risk ratings were positively correlated with left insula ($r(76)= .23$, $p = .043$), left inferior parietal lobule ($r(76)= .24$, $p = .035$), and there was a positive trend in left thalamus ($r(76)= .20$, $p = .073$). No other fROI correlations were significant (p-values $\geq$ .12). Risk ratings were not significantly correlated with CAPS scores or other indices of symptom severity (p-values $\geq$ .24).

Connectivity Results

Results from PPI analyses are listed in table 3.3. Left and right hippocampus served as seeds for PPI analyses based on importance of the hippocampus in the neural model of generalization proposed by Lissek and colleagues (2013). With the right hippocampus as the seed, functional coupling with the left inferior parietal lobule, right anterior cingulate, left thalamus, and dmPFC were stronger during CS+ versus vCS- (*p*-values < .05, uncorrected). Using the left hippocampus as the seed, greater functional coupling to CS+ versus vCS- was found in bilateral insula and right thalamus (*p*-values < .05, uncorrected). Greater functional coupling to vCS- versus CS+ was found between the left hippocampus and the right precuneus and vmPFC (*p*-values < .05, uncorrected).

Table 3.3
Results from psychophysiological interaction analyses

Seed Region	**Target Region**	**Effect**	**Peak Coordinates***			***t*-value**
			X	**Y**	**Z**	
R Hippocampus	IPL	CS+ > vCS-	45.0	42.0	38.5	4.06
	R ACC	CS+ > vCS-	-9.0	-21.0	25.0	3.81
	L Thal	CS+ > vCS-	18.0	12.0	10.0	5.09
	dmPFC	CS+ > vCS-	-7.5	-42.0	25.0	4.10
L Hippocampus	R PCu	vCS- > CS+	-27.0	72.0	25.0	4.21
	R Thal	CS+ > vCS-	-9.0	31.5	10.0	7.05
	vmPFC	vCS- > CS+	-6.0	-46.5	-11.0	6.69
	R	CS+ > vCS-	-33.0	-9.0	-3.5	4.91
	L	CS+ > vCS-	27.0	28.5	20.5	4.85

Note. vCS- = v-shaped conditioned safety cue; CS+ = conditioned danger cue; L = left; R = right; IPL = inferior parietal lobule; ACC = anterior cingulate cortex; Thal = thalamus; dmPFC = dorsal medial prefrontal cortex; PCu = precuneus; vmPFC = ventral medial prefrontal cortex. All effects were significant at $p \leq .05$, uncorrected. *Peak coordinates are in RAI format.

Discussion

This study found that PTSD patients showed evidence of overgeneralization of conditioned fear in bilateral insula, dmPFC, and right and left caudate. Specifically, PTSD patients showed less steep, linear declines in BOLD activation as the presented stimulus becomes less similar to CS+, suggesting heightened activation of fear-related brain areas to stimuli that approximate the conditioned danger cue. Consistent with previous research in healthy participants (Lissek et al., 2013), non-trauma controls, trauma controls, and the sub-threshold PTSD group showed steep, quadratic declines in BOLD activations in bilateral insula, dmPFC, and right and left caudate, suggesting a rapid decline in activation of fear-related brain areas as the stimulus diverged in similarity to CS+. Also consistent with Lissek et al., (2013), the current study found that bilateral hippocampus, vmPFC, and precuneus showed the greatest activation to the CS- in all participants, which then decreased gradually with increasing similarity to the conditioned danger cue. No group differences were apparent in these brain regions in the current study.

Behavioral results also showed declines in risk ratings as the stimulus becomes less similar to CS+, consistent with previous work (Lissek et al., 2010; Lissek et al., 2013). Additionally, PTSD patients and the sub-threshold PTSD group generalized perceived risk to rings with two degrees of differentiation from CS+ (GS_2 and GS_3) while the trauma and non-trauma controls only generalized to rings with one degree of differentiation (GS_3), suggesting overgeneralization in the PTSD and sub-threshold PTSD groups. Skin conductance results also showed declines in skin conductance as the

stimulus decreases in similarity to CS+, although the gradients were similar across groups.

Evaluation of a neurobiological model of conditioned fear generalization. The results of this study support the neurobiological model of conditioned fear generalization proposed by Lissek and collegues (Lissek et al., 2013). According to this model, the sensory cortex and the hippocampus assist in pattern-matching between the encountered stimulus and previous learned conditioned danger cues and this information is then relayed to brain areas associated with fear excitation (e.g., insula) and fear inhibition (e.g., vmPFC). Animal research implicates the hippocampus in generalization of fear conditioning, with lesions of the hippocampus or its cortical inputs leading to greater fear generalization in animals (Bucci et al., 2002; Solomon & Moore, 1975; Wild & Blampied, 1972). The hippocampus is also implicated in schematic matching which is necessary for discrimination of CS+ from CS- (O'Reilly & Rudy, 2001; Otto & Eichenbaum, 1992; Sander, Grandjean, & Scherer, 2005).

Likewise, the sensory regions of the brain are proposed to contribute to conditioned generalization by processing stimulus features of CS+ and GSs, which is supported by increased generalization of conditioned fear following lesions of the sensory cortex and thalamus in animals (Antunes & Moita, 2010; Jarrell et al., 1987; Teich et al., 1988; for opposing results see Armony et al., 1997). The results of the current study support the importance of the hippocampus and sensory regions including the thalamus in schematic matching, as evidenced by decreases in activation in these areas as the stimulus becomes more similar to the danger cue. However, we did not find

evidence of group differences in hippocampal or sensory region activation, suggesting that PTSD patients did not experience deficits in pattern separation in this study.

Fear-related brain regions. Fear-related brain areas that are proposed to be mediated by the hippocampus and sensory regions include the insula, dmPFC, and amygdala. Activation of bilateral insula to CS+ in the current study is consistent with previous work showing the importance of the insula in anticipatory processing, fear conditioning, and salience appraisal (Lissek et al., 2013; Lovero et al., 2009; Menon and Uddin, 2010; Nitschke, Sarinopoulos, Mackiewicz, Schaefer, & Davidson, 2006; Paulus & Stein, 2006). The insula is thought to integrate threat information and transmits it to brain areas responsible for attentional control, physiological arousal, and motor responding. The current study was able to demonstrate less steep, linear declines in BOLD activation as the presented stimulus becomes less similar to CS+ in PTSD patients compared to quadratic declines in controls, suggesting that overgeneralization of conditioned fear is a neurobiological marker of psychopathology to trauma.

Another region implicated during fear excitation is the dmPFC. Animal studies demonstrate that lesions of the dmPFC result in greater generalization of conditioned fear to contextual stimuli in rats (Morgan & LeDoux, 1995). Furthermore, the dmPFC has been implicated in the appraisal and expression of negative emotion (Etkin, Egner, & Kalisch, 2011) and in fear conditioning responses (Lissek et al., 2013). The current study found declines in BOLD activation as the presented stimulus becomes less similar to CS+, consistent with previous research (Lissek et al., 2013). Additionally, PTSD patients

showed less steep, linear declines in the dmPFC, suggesting greater generalization in this group.

Conversely, this study was unable to find differences in amygdala activation for the CS+ vs. vCS- contrast using the current paradigm. The amygdala is associated with fear acquisition and expression (Davis, 1992; LeDoux, 2000). Previous work using fear conditioning paradigms suggests that amygdala responses habituate with increasing number of trials (Büchel, Morris, Dolan, & Friston, 1998); however, even when restricting analyses to the first half of the generalization trials, there was still no differential amygdala activation to CS+ vs. vCS- in this study. Others have also reported a lack of amygdala activation between CS+ and GS or CS-comparisons (Greenberg et al., 2013; Lissek et al., 2013). Greenberg et al. (2013) noted that the similar levels of amygdala activation to CS+ and GSs may be due to the similarity between CS+ and GSs or may represent engagement of the amygdala to all stimuli while the relationship between the stimulus and shock is learned. The current study employed a safety cue that was dissimilar in shape to the danger cue; therefore, the absence of a differential response in the amygdala cannot be explained by similarity between CS+ and vCS-. However, because CS+, oCS-, and vCS- were presented together during the acquisition phase, it is possible that there is amygdala engagement to all stimuli that were presented during conditioning.

Brain areas associated with prediction of appetitive/aversive stimuli. This study was able to demonstrate activation of the left and right caudate to CS+ versus vCS-, consistent with the work by Greenberg and colleagues. Activation of the dorsal striatum

(consisting of the caudate and putamen) has been found to be associated with reward prediction (for a review, see O'Doherty, 2004) as well as prediction of negative outcomes, such as shock or monetary loss (for a review, see Delgado, Li, Schiller, & Phelps, 2008). In animal research, lesions to the dorsal striatum have been shown to lead to abnormalities in conditioned freezing, conditioned emotional responses, and passive and active avoidance (e.g., Winocur, 1974; Prado-Alcala et al., 1975; Viaud & White, 1989; White & Salinas, 2003; for an overview, see Delgado et al., 2008). Activation of the striatum has also been shown in humans during fear conditioning (Büchel, Dolan, Armony, & Friston, 1999; LaBar et al. 1998; Phelps et al. 2004; Schiller et al., 2008) and during an instructed threat generalization paradigm (Greenberg et al., 2013a; 2013b). In particular, activations of the striatum and especially the caudate have been shown to be correlated with prediction error (PE) signals, which arise when an outcome deviates from expectation (Delgado et al., 2008). Greenberg et al. (2013a) note that we would expect to see a gradual decline in PE signals and associated caudate activation during generalization because the expectation of receiving a shock decreases as the stimulus becomes less similar to CS+.

Regions associated with fear inhibition. In addition to fear related brain areas, information from the sensory regions and hippocampus is also proposed to be relayed to regions associated with the inhibition of fear such as the ventral medial prefrontal cortex (vmPFC; Lissek et al., 2013). The vmPFC is implicated in the inhibition of fear responses. Animal studies show that lesions in the vmPFC lead to the generalization of conditioned fear in rats to unpaired contexts and contextual stimuli (Morgan & LeDoux,

1995; Zelinski et al., 2010). The importance of the vmPFC in fear inhibition is further supported by research in humans showing activation of the vmPFC to conditioned stimuli following extinction training (Milad et al., 2007; Phelps et al., 2004; Schiller et al., 2008). As predicted by the neurobiological model of generalization proposed by Lissek and colleagues, the results of this study showed the greatest activation to the CS- in vmPFC, which then decreased gradually with increasing similarity to CS+. However, the current study did not find group differences in activation of the vmPFC.

Inhibitory vs. excitatory fear conditioning accounts of PTSD. Two processes that have been proposed to lead to enhanced fear responding in PTSD patients during fear conditioning include deficits in inhibition and excessive excitation of fear-related networks. Davis and colleagues (2000) and Jovanovic and Ressler (2010) proposed that the impaired ability to inhibit a conditioned fear response leads to enhanced fear responding to safety cues. Conversely, excitation accounts of fear conditioning posit that enhanced fear responses are the result of excessive excitation of fear-related networks which leads to overgeneralization of conditioned fear (Lissek & Grillon, 2012).

Functional imaging methods can be used to dissociate excitatory and inhibitory processes by examining whether abnormalities in conditioned fear are associated with abnormalities in excitatory brain mechanisms (e.g., insula) or inhibitory ones (e.g., vmPFC). The results of the current study demonstrated enhanced fear responding to stimuli that approximate the conditioned danger cue in PTSD patients compared to controls in bilateral insula and dmPFC but no group differences were found in vmPFC. Enhanced fear responding in this experimental paradigm thus seems to have been due to

excessive excitation rather than impaired inhibition, suggesting that PTSD is associated with abnormalities in excitatory neural networks. However, symptoms of PTSD are likely due to a complex interaction between excitatory and inhibitory processes and further imaging research is needed using fear inhibition paradigms.

Limitations and future directions. A limitation of the current study includes the use of males with primarily combat-related trauma, which was the result of recruitment through a Veteran's Affairs Medical Center. The results of this study require replication in females and those with diverse trauma experiences such as sexual or physical assaults, accidents, or natural disasters. An additional limitation includes the insufficient number of unreinforced CS+ trials during the acquisition phase, which did not allow for analysis of group differences during acquisition training. Future studies on conditioned fear generalization in PTSD could focus on whether generalization differs based on the type of trauma experienced or whether time since the trauma has an effect as we might expect overgeneralization to decrease with time as the severity of PTSD symptoms decreases.

Chapter 4: Implications of the research on conditioned fear generalization in PTSD and OCD

This dissertation attempted to fill a gap in the fear conditioning literature by investigating the generalization of conditioned fear in PTSD and OCD. A review of the literature demonstrated a lack of research on fear-conditioning processes in OCD. Although those with OCD have been suggested to overgeneralize threat; no studies to date have investigated conditioned fear generalization processes in OCD. Chapter 2 represents the first study that attempted to investigate the generalization of conditioned fear in individuals with obsessive-compulsive traits using startle EMG. The results of this study demonstrated that individuals with high levels of Threat Estimation as measured by the Obsessive Beliefs Questionnaire (OBQ-44) displayed overgeneralization of fear responses to a greater range of stimuli resembling the danger cue than those with low levels of Threat Estimation. In particular, the high Threat Estimation group showed greater fear-potentiated startle to ring sizes up to two units of dissimilarity from the danger cue while the low Threat Estimation group did not generalize the conditioned fear response beyond the danger cue. This suggests that the high Threat Estimation group may be characterized by lower thresholds of threat reactivity, which results in greater fear responses to stimuli that resemble the danger cue.

In addition, despite etiological theories proposing that fear conditioning and overgeneralization of fear play prominent roles in the development and maintenance of PTSD, little research had been done the neurobiological mechanisms that contribute to fear conditioning processes in PTSD patients. Chapter 3 investigated the neurobiological

substrates associated with the overgeneralization of conditioned fear in PTSD patients using skin conductance measures and functional magnetic resonance imaging (fMRI). This study provided evidence of overgeneralization of conditioned fear in PTSD patients in bilateral insula, dmPFC, and right and left caudate.

The implications of these studies bring to bear several important points which merit further discussion. First, the lack of a significant difference in those with and without OC traits in terms of generalization brings to light the obvious but important point that fear conditioning processes may not be relevant to all anxiety disorders. Second, these findings also suggest that identification of processes related to the generalization of conditioned fear that are common across anxiety disorders (such as overestimation of threat) may be beneficial to our understanding of generalization. Third, the differences in neural functioning found during conditioned fear between PTSD patients and controls raises the question of whether these differences reflect a risk factor or a consequence of the disorder. Last, the need for an integrative model of anxiety that incorporates non-associative learning processes will be discussed.

Relevancy of Fear Conditioning Processes to PTSD and OCD

Of the anxiety disorders, PTSD appears to have the most straightforward relationship with fear-conditioning processes. Based on the direct relationship between a traumatic event and the subsequent fear of trauma-relevant stimuli, the etiology of PTSD seems well accounted for by a fear conditioning model. In support of the fear conditioning model of PTSD, chapter 3 demonstrated the overgeneralization of conditioned fear in brain regions such as the insula, dmPFC, and right and left caudate in

PTSD patients compared to controls. Future directions in PTSD research would benefit from studies investigating the contribution of other associative fear learning theories to the maintenance of this disorder. For example, fear conditioning processes that have been understudied in PTSD include avoidance, extinction retention, and associative learning deficits.

The current body of work also predicted that OCD would be characterized by overgeneralization; however, chapter 2 was only able to find evidence of overgeneralization in those high in Threat Estimation as measured by the OBQ-44. No differences in generalization were found when comparing individuals with high and low levels of overall obsessive-compulsive symptoms as measured by the Obsessive-compulsive Inventory-Revised (OCI-R). A noted limitation to this study was use of a non-clinical population of individuals with obsessive-compulsive traits rather than patients with OCD. Overgeneralization of conditioned fear may be more apparent in a study using clinical patients with OCD.

As mentioned previously, the lack of group differences in the high and low obsessive-compulsive groups may also be due to the lack of disorder-relevant conditioned stimuli used in this study's experimental paradigm. It has been noted that paradigms that use threat of shock as the unconditioned stimulus may not be as pertinent to anxiety disorders where threat of physical harm is not a key feature (Lissek, Pine, & Grillon, 2006). Fear of physical harm is thought to be characteristic of posttraumatic stress disorder but may be less relevant to obsessive-compulsive disorder. Experimental paradigms that use OCD-specific unconditioned stimuli such as contamination may be

more successful at finding group differences in fear responses in obsessive-compulsive populations.

However, it is also possible that overgeneralization of conditioned fear is more relevant to particular anxiety disorders that are conceptualized as originating from fear conditioning processes such as PTSD, rather than OCD. Theoretically, fear conditioning accounts of OCD are underdeveloped and there is currently not enough empirical research on conditioned fear in these disorders to draw any conclusions about the merit, or lack thereof, of using fear conditioning processes to study OCD symptoms. In general, OCD is not conceptualized as originating from conditioned fear. Associative fear learning theories are based on the assumption that in order for fear conditioning to occur, there has to be an opportunity to associate stimuli with an aversive event. However, in the case of OCD, many patients appear to develop a fear response to a stimulus without recollection of an aversive experience.

However, this does not mean that fear conditioning paradigms cannot be useful in studying this disorder. For example, those with OCD may show abnormalities in conditioned fear processes which provide valuable information about how fear is processed in this disorder. In particular, greater avoidance (via compulsions) leading to failure to extinguish fear may be especially relevant to OCD. The efficacy of exposure and response prevention therapy for OCD, a treatment modality that aims to extinguish the fear response, supports the association between OCD and fear conditioning processes such as extinction. Additional research using fear conditioning paradigms with OCD

patients is necessary before inferences can be made about the role of fear conditioning processes in the maintenance of this disorder.

Relation of Conditioned Fear Generalization to Processes Common Across Disorders

Most studies on the generalization of conditioned fear focus on healthy participants or disorder-specific group differences. Although this approach has advanced our understanding of generalization in different anxiety disorders, an advantageous next step may be to identify processes related to the generalization of conditioned fear that are common across anxiety disorders (such as overestimation of threat). Knowledge about how anxiety disorders differ from one another and from healthy participants is important and valid work. But understanding the underlying processes that lead to overgeneralization may help elucidate why some anxiety disorders show overgeneralization while others do not.

For example, chapter 2 did not find differences in generalization between individuals with high and low levels obsessive-compulsive symptoms as measured by the Obsessive-compulsive Inventory-Revised (OCI-R). Instead, this study found that overgeneralization was restricted to the high in Threat Estimation group as measured by the OBQ-44. As mentioned previously, the finding of greater generalization in the high Threat Estimation group compared to the low Threat Estimation group and no differences in the high and low obsessive-compulsive groups may be due to overestimation of threat being non-specific to OCD. It has been shown that threat estimation does not reliably

differentiate OCD patients from other anxiety patients (Anholt et al., 2006; Clark, Purdon, & Wang, 2003; Sica et al., 2004; Tolin, Worhunsky, & Maltby, 2006).

Overestimation of threat is implicated in many anxiety disorders including GAD, PTSD, and panic disorder. Excessive threat estimation may be one process common across many anxiety disorders that contributes to the overgeneralization of conditioned fear. Furthermore, differences in generalization between anxiety groups may reflect differences in the levels of threat estimation between each disorder which would explain why some anxiety disorders show overgeneralization while others do not. For example, perhaps PTSD patients show a greater propensity to overestimate threat than OCD patients, which would explain why PTSD patients and not those with OC traits show overgeneralization of conditioned fear. Hypotheses such as these require further investigation. Although research investigating processes common across anxiety disorders that underlie the generalization of conditioned fear is in its infancy, it provides a promising area for future work.

Overgeneralization: Risk Factor or Result of the Disorder?

Chapter 3 demonstrated evidence of overgeneralization in PTSD patients compared to controls in bilateral insula, dmPFC, and right and left caudate which raises the question of whether these differences reflect a risk factor for developing pathological anxiety or whether they are a consequence of the disorder. In other words, impaired mechanisms of conditioned fear generalization may contribute to the development of anxiety disorders such as PTSD (risk factor) or deficits may arise from the anxiety disorder itself (acquired trait). There is some evidence that pre-trauma hypersensitivity to

contextual threat, enhanced fear responses to explicit threat, and failure to habituate to aversive stimuli are predictive of post-trauma symptoms of PTSD (Pole et al., 2009), suggesting the existence of pre-morbid risk factors for PTSD. Conversely, a twin study found that deficits in extinction retention in PTSD patients were not present in their co-twins, suggesting these deficits were acquired post-trauma rather than representing a vulnerability factor (Milad et al., 2008). Future work would benefit from prospective studies focusing on the generalization of conditioned fear in order to determine whether greater generalization predicts an increased risk for anxiety disorders such as PTSD.

However, it is likely a complex interaction of risk factors (genetic, environmental, neurobiological, etc.) that influences the manifestation of pathological anxiety. For example, not all individuals who experience a traumatic event will develop PTSD; rather, a vulnerability to developing an anxiety disorder is also required. Additional research is needed on the specific vulnerabilities that influence conditioned fear processes such as generalization. We do know that individual differences in the development of conditioned fear are influenced by past experience. Specifically, non-fearful encounters experienced either directly or vicariously can hinder the development of conditioned fear. For example, animal research demonstrates that rhesus monkeys that observe other monkeys responding non-fearfully to a snake are less likely to develop conditioned fear to snakes (Mineka & Cook, 1986; 1993). Specific vulnerabilities and the protective effects of experience explain why not all aversive experiences result in the development of an anxiety disorder. The exact nature of the vulnerabilities that lead to the development of

abnormalities in fear conditioning processes is another area in need of additional research.

Beyond Fear Conditioning: the Need for an Integrative Model of Anxiety

An integrative model of anxiety that incorporates both associative and non-associative learning processes is needed in order to provide a more comprehensive understanding of the processes that lead to the development and maintenance of pathological anxiety. Critics of conditioned fear theories argue that fear conditioning processes are not the only means by which pathological anxiety can be developed and maintained. In contrast to fear conditioning accounts of pathological anxiety, others have suggested the importance of non-associative learning mechanisms in the maintenance of anxiety. While associative learning accounts focus the association of a neutral stimulus with a positive or negative outcome that influences behavioral responses, non-associative learning theories are based on the idea that changes in a behavioral response to a stimulus occur in the absence of positive or negative outcomes.

Two prominent non-associative learning accounts include habituation and sensitization. Habituation refers to a decrease in a behavioral response to a stimulus over time following repeated exposure to that stimulus, while sensitization refers to an increase in a behavioral response with repeated exposure to a stimulus. Deficits in the ability to habituate to feared stimuli and/or increased sensitization to feared stimuli are important but understudied contributors to pathological anxiety. Habituation and sensitization are not incompatible with fear conditioning theories and it is likely that deficits in habituation and excessive sensitization interact with fear conditioning

processes in the maintenance of pathological anxiety. Future research on pathological anxiety would benefit from additional studies designed to measure non-associative learning principles. However, research paradigms are needed that can disentangle the relative contributions of associative and non-associative learning processes.

Conclusion

In conclusion, this body of work aimed to address the lack of studies on the generalization of conditioned fear in OCD and PTSD. The results discussed in chapter 2 suggest that the generalization of conditioned fear may not be relevant to all anxiety disorders based on the lack of a significant difference in those with and without OC traits. Future work using clinical patients with OCD and/or OCD-specific unconditioned stimuli to explore generalization effects may better be able to find group differences. However, this study was able to demonstrate greater generalization in those who overestimate threat and it was suggested that the identification of processes related to the generalization of conditioned fear that are common across anxiety disorders (such as overestimation of threat) may refine our understanding of generalization effects. Additionally, future research would benefit from exploring other fear conditioning processes that may be especially relevant to OCD such as extinction.

Furthermore, chapter 3 demonstrated that PTSD patients shown overgeneralization of conditioned fear in bilateral insula, dorsal medial prefrontal cortex, and right and left caudate. It remains unclear whether these differences in neural functioning represent a risk factor or a consequence of the disorder; therefore it was suggested that prospective studies focusing on the generalization of conditioned fear be

conducted in order to determine whether greater generalization predicts an increased risk for anxiety disorders such as PTSD. Additionally, future directions in PTSD research would benefit from studies investigating other understudied associative fear learning theories such as avoidance, extinction retention, and associative learning deficits. And finally, the refinement of current theoretical accounts of pathological anxiety with the integration associative learning theories with non-associative learning processes (e.g., habituation, sensitization) is needed in order to provide a more comprehensive understanding of the processes that lead to the development and maintenance of pathological anxiety.

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